The Effect of Maturity Stage of White-Brined Cheese on the Consistency of Processed Cheese

Bc. Milan Bartoš

Univerzita Tomáše Bati ve Zlíně
Fakulta technologická
Ústav technologie potravin
akademický rok: 2016/2017

ZADÁNÍ DIPLOMOVÉ PRÁCE
(PROJEKTU, UMĚLECKÉHO DÍLA, UMĚLECKÉHO VÝKONU)

Jméno a příjmení:　**Bc. Milan Bartoš**
Osobní číslo:　**T15273**
Studijní program:　**N2901 Chemie a technologie potravin**
Studijní obor:　**Technologie potravin**
Forma studia:　**prezenční**

Téma práce:　**Vliv doby prozrálostí vybraných sýrů zrajících v solném nálevu na konzistenci tavených sýrů**

Zásady pro vypracování:

I. Teoretická část

1. Stručně charakterizujte výrobu a vlastnosti tavených sýrů.

2. Popište mechanizmus působení fosforečnanových a citronanových tavicích solí v tavených sýrech.

3. Charakterizujte vliv prozrálosti suroviny a podmínek skladování na vlastnosti tavených sýrů.

4. Stručně popište výrobu bílých sýrů zrajících v solném nálevu.

II. Praktická část

1. Vyrobte modelové tavené sýry, kde základní surovinou bude sýr zrající v solném nálevu v různém stádiu prozrálosti.

2. Při výrobě modelových tavených sýrů použijte ternární směsi sodných solí fosforečnanů a polyfosforečnanů.

3. Modelové vzorky skladujte 60 dnů a v průběhu skladování proveďte měření hodnot pH, texturní profilové analýzy a viskoelastických vlastností modelových vzorků.

4. Výsledky vyhodnoťte a formulujte závěry.

Rozsah diplomové práce:

Rozsah příloh:

Forma zpracování diplomové práce: **tištěná/elektronická**

Seznam odborné literatury:

[1] SALEK, R. N., ČERNÍKOVÁ, M., NAGYOVÁ, G., KUCHAŘ, D., BAČOVÁ, H., MINARČÍKOVÁ, L. a 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 [online]. 2015, vol. 44, s. 37–43 [cit. 2015-04-15]. DOI: 10.1016/j.idairyj.2014.12.009.
[2] KAPOOR, R. and METZGER, L. E., Process cheese: Scientific and technological aspects A Review. Compr. Rev. Food Sci. Food Saf., 2008, s. 194–214. ISSN 1541-4337.
[3] CUNHA, C. R., DIAS, A. I. and VIOTTO, W. H. Microstructure, texture, colour and sensory evaluation of a spreadable processed cheese analogue made with vegetable fat. Food Research International, 2010, 43, s. 723729. ISSN 0963-9969.
[4] BRICKLEY, C. A. and GOVINDASAMY–LUCEY, S. Influence of emulsifying salts on the textural properties of nongat process cheese made from direct acid cheese bases, Journal of Dairy Science, 2008, 91, s. 3948. ISSN 0022-0302.
[5] SHIRASHOJI, N., JAEGGI, J. J. and LUCEY, J. A. Effects of various emulsifying salts on the rheological and textural properties of pasteurized process Chedar cheese. Journal of Dairy Science, 2005, 88. s. 363 369. ISSN 0022-0302.

Vedoucí diplomové práce: **Ing. Richardos Nikolaos Salek**
Ústav technologie potravin

Datum zadání diplomové práce: **3. února 2017**

Termín odevzdání diplomové práce: **28. dubna 2017**

Ve Zlíně dne 3. února 2017

doc. Ing. František Buňka, Ph.D.
děkan

L.S.

doc. Ing. František Buňka, Ph.D.
ředitel ústavu

Příjmení a jméno: Milan Bartoš

Obor: Technologie potravin

PROHLÁŠENÍ

Prohlašuji, že

- beru na vědomí, že odevzdáním diplomové/bakalářské práce souhlasím se zveřejněním své práce podle zákona č. 111/1998 Sb. o vysokých školách a o změně a doplnění dalších zákonů (zákon o vysokých školách), ve znění pozdějších právních předpisů, bez ohledu na výsledek obhajoby [1];
- beru na vědomí, že diplomová/bakalářská práce bude uložena v elektronické podobě v univerzitním informačním systému dostupná k nahlédnutí, že jeden výtisk diplomové/bakalářské práce bude uložen na příslušném ústavu Fakulty technologické UTB ve Zlíně a jeden výtisk bude uložen u vedoucího práce;
- byl/a jsem seznámen/a s tím, že na moji diplomovou/bakalářskou práci se plně vztahuje zákon č. 121/2000 Sb. o právu autorském, o právech souvisejících s právem autorským a o změně některých zákonů (autorský zákon) ve znění pozdějších právních předpisů, zejm. § 35 odst. 3 [2];
- beru na vědomí, že podle § 60 [3] odst. 1 autorského zákona má UTB ve Zlíně právo na uzavření licenční smlouvy o užití školního díla v rozsahu § 12 odst. 4 autorského zákona;
- beru na vědomí, že podle § 60 [3] odst. 2 a 3 mohu užít své dílo – diplomovou/bakalářskou práci nebo poskytnout licenci k jejímu využití jen s předchozím písemným souhlasem Univerzity Tomáše Bati ve Zlíně, která je oprávněna v takovém případě ode mne požadovat přiměřený příspěvek na úhradu nákladů, které byly Univerzitou Tomáše Bati ve Zlíně na vytvoření díla vynaloženy (až do jejich skutečné výše);
- beru na vědomí, že pokud bylo k vypracování diplomové/bakalářské práce využito softwaru poskytnutého Univerzitou Tomáše Bati ve Zlíně nebo jinými subjekty pouze ke studijním a výzkumným účelům (tedy pouze k nekomerčnímu využití), nelze výsledky diplomové/bakalářské práce využít ke komerčním účelům;
- beru na vědomí, že pokud je výstupem diplomové/bakalářské práce jakýkoliv softwarový produkt, považují se za součást práce rovněž i zdrojové kódy, popř. soubory, ze kterých se projekt skládá. Neodevzdání této součásti může být důvodem k neobhájení práce.

Ve Zlíně, 9.5.2017

....

(3) Do práva autorského také nezasahuje škola nebo školské či vzdělávací zařízení, užije-li nikoli za účelem přímého nebo nepřímého hospodářského nebo obchodního prospěchu k výuce nebo k vlastní potřebě dílo vytvořené žákem nebo studentem ke splnění školních nebo studijních povinností vyplývajících z jeho právního vztahu ke škole nebo školskému či vzdělávacího zařízení (školní dílo).

3) zákon č. 121/2000 Sb. o právu autorském, o právech souvisejících s právem autorským a o změně některých zákonů (autorský zákon) ve znění pozdějších právních předpisů, § 60 Školní dílo:

(1) Škola nebo školské či vzdělávací zařízení mají za obvyklých podmínek právo na uzavření licenční smlouvy o užití školního díla (§ 35 odst. 3). Odpírá-li autor takového díla udělit svolení bez vážného důvodu, mohou se tyto osoby domáhat nahrazení chybějícího projevu jeho vůle u soudu. Ustanovení § 35 odst. 3 zůstává nedotčeno.

(2) Není-li sjednáno jinak, může autor školního díla své dílo užít či poskytnout jinému licenci, není-li to v rozporu s oprávněnými zájmy školy nebo školského či vzdělávacího zařízení.

(3) Škola nebo školské či vzdělávací zařízení jsou oprávněny požadovat, aby jim autor školního díla z výdělku jím dosaženého v souvislosti s užitím díla či poskytnutím licence podle odstavce 2 přiměřeně přispěl na úhradu nákladů, které na vytvoření díla vynaložily, a to podle okolnosti až do jejich skutečné výše; přitom se přihlédne k výši výdělku dosaženého školou nebo školským či vzdělávacím zařízením z užití školního díla podle odstavce 1.

ABSTRAKT

Cílem diplomové práce byla studie vlivu doby prozrálosti vybraných sýrů zrajících v solném nálevu na konzistenci tavených sýrů s různou skladbou tavicích solí v průběhu 60 denního skladování. Pro výrobu modelových vzorků tavených sýrů byla použita 2, 4, 8, 16 a 24 týdenní surovina. Jako tavicí soli byly použity citran sodný (TSC), hydrogenfosforečnan sodný (DSP), difosforečnan sodný (TSPP) a sodná sůl polyfosforečnanu (P20). Z těchto tavicích solí byly vytvořeny 4 kombinace ternárních směsí tavicích solí (DSP:TSC:P20, DSP:TSPP:TSC, TSC:TSPP:P20 a DSP:TSPP:P20).

Modelové vzorky tavených sýrů byly podrobeny základní chemické analýze (stanovení obsahu sušiny a hodnot pH) a texturní profilové analýze (tvrdost, relativní lepivost a kohezivnost). V experimentální části byl pozorován rostoucí trend tvrdosti modelových vzorků tavených sýrů v průběhu skladování, naopak v závislosti na prozrálosti použité suroviny tvrdost vzorků klesala. Při stanovení tvrdosti výrobku byl pozorován specifický jev při aplikaci TSC:TSPP a DSP:TSPP v poměru 1:1 s nulovou koncentrací P20.

Klíčová slova: tavený sýr, sýr zrající v solném nálevu, tavicí soli, citran sodný, fosforečnany, tvrdost

ABSTRACT

The aim of the Master's thesis was to study the effect of the maturity stage of Akawi white-brined cheese on the consistency of processed cheese model samples with different composition of emulsifying salts during a 60-day storage period. For the manufacture of the model processed cheese samples was used cheese with 2, 4, 8, 16 and 24 weeks of maturity as the main raw ingredient. Trisodium citrate (TSC), disodium hydrogenphosphate (DSP), tetrasodium diphosphate (TSPP) and sodium salt of polyphosphate (P20) were used as emulsifying salts. Moreover, of these emulsifying salts, 4 combinations of ternary mixtures of emulsifying salts (DSP:TSC:P20, DSP:TSPP:TSC, TSC:TSPP:P20 and DSP:TSPP:P20) were created.

The model samples of processed cheeses have been subjected to basic chemical analysis (including determination of dry matter content and pH values, respectively) and textural profile analysis (hardness, relative adhesiveness and cohesiveness). In the experimental part, the increasing trend of hardness of processed cheese model samples was observed during storage. On the other hand, depending on the maturity of the cheese (used as raw material), the hardness of the samples decreased. The specific effect of TSC:TSPP and DSP:TSPP in the ratio 1:1 with zero concentrations of P20 was observed during the determination of the model samples hardness.

Keywords: Processed cheese, White-brined cheese, emulsifying salts, sodium citrate, phosphates, hardness

ACKNOWLEDGEMENTS

Heartily I would like to express my acknowledgements to Ing. Richardos Nikolaos Salek, Ph.D., for the guidance and the support that had offered me throughout the length of this Master's thesis. His interest and patience were irreducible both during the realization of the experiment and its writing. Without his help the accomplishment of this work would not be possible.

Finally I would like to express my acknowledgements to all those that had cooperated for the accomplishment of this Master's thesis and I forgot to mention them.

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

CONTENTS

INTRODUCTION

The technology of processed cheese production is one of the youngest production processes in the dairy industry. Processed cheeses (PC) are milk products made from natural cheeses of varying degrees of maturity and heat-treated with the addition of butter, water, emulsifying salts (ES) and other dairy and non-dairy ingredients. The presence of ES is essential for the formation of a homogeneous structure. The basic role of ES is ion exchange of calcium ions in the milk protein of natural cheese for sodium ions. The first mention of the production of PC with the addition of ES dates back to 1911 from Gerber company. PC are a favorite commodity due to their versatility of products and the possibilities of using them both for households, restaurants and fast foods [1, 2].

The theoretical part of the Master's thesis includes four chapters. The first chapter discusses the characteristics of PC, their production and main ingredients using during processing. The second chapter focuses on key ingredients for the production of PC which are ES. Phosphate- and citrate-based emulsifying salts are described here, in particular their basic role in the production of PC. The third chapter focuses generally on the factors affecting the consistency of PC, especially the influence of the natural cheeses on the consistency of PC. The last chapter describes White-brined natural cheeses and the ripening process of these cheeses.

The aim of the experimental part of this Master's thesis was to study the influence of maturity stage of White-brined natural cheeses on the consistency of PC using various ES compositions. Basic chemical and texture analysis of the samples was performed. The experimental part of the Master's thesis included the production of PC model samples. White-brined natural Akawi cheese has been used with different stages of maturity. As the ES disodium hydrogenphosphate (DSPP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate (P20) and trisodium citrate (TSC) were used. From these types of ES, 4 combinations of ternary mixtures were created. The experimental part was performed for 60 days at regular intervals (2nd, 9th, 30th and 60th day of storage). The experimental part summarizes and discusses the results of pH measurement, dry matter (DM) content and selected textural properties (hardness, cohesion and relative adhesiveness) of PC.

I. THEORY

1 PROCESSED CHEESE

PC are currently one of the most popular and widespread group of dairy products. According to the Czech Statistical Office, consumption of PC was 2.0 kg/per capita in the Czech Republic in 2015. It is almost double in comparison with other countries of the European Union. The high demand of PC can be found due to affordability and extended shelf-life primarily, compared to other dairy products. Variability of textural properties, fat content (FC), taste, aroma, packaging size and shape, makes it ideal for the various options for application in both warm and cold dishes. However, the demand of PC slightly decreased compared to year 2014 (2.1 kg/per capita), because of the increasing popularity of PC analogues [1, 2].

PC is heat-treated cheese with the addition of ES. Minimum of DM content is at least 51 % (w/w) and must origin from natural cheese. Moreover, if it contains more than 5 % (w/w) of lactose, can not be labeled as PC, acording to Ministry of Agriculture Decree 397/2016 Coll [3].

Moreover, acording to the Codex Alimentarius, PC are dairy products obtained from natural cheese (with or without the addition of other raw materials and permitted ingredients) by melting and emulsifying the mixture with the aid of heat and emulsifying (or melting) salts in a well-mixed medium to produce a homogeneous, smooth and stable oil-in-water emulsion [4].

Additionally, PC can be characterized as a viscoelastic matrix. The basic material consists of natural cheese/cheeses at different stages of maturity. It is made by using a wide range of dairy (e.g., cream, butter, anhydrous milk fat, curd, milk powder, whey powder, caseinates) and non-dairy ingredients and additives (e.g. hydrocolloids, coloring, sensory active agents) which are applied in order to modify the content (e.g. DM content, FC, protein content) or the functional properties of the end-product (e.g. firmness, meltability). PC are manufactured in many countries, offering numerous variants that have different denominations such as *"pasteurized"*, *"emulsified"*, *pasteurized blended*, *"American"*, *"cooked"* or *"sterilized"* cheese. In addition, PC products are available in many different versions including blocks, slices, and individually packaged portions such as snacks, cups and tubes [5 – 7].

1.1 Ingredients of dairy origin

PC are not made from milk directly, whereas are based on natural cheese/cheeses in various stages of maturity. This is the main difference between processed and natural cheeses. Natural cheeses with mechanical defects can be used in the production of PC, because appearance of cheese with defects can be inappropriate for consumers. Cheese with microbiological defects shall not be used for processing. Especially cheese conteining spore-forming bacteria or fungi. The most common spore-forming bacteria found in dairy products belong to the genera *Bacillus* and *Clostridium*, which contain several species that are of concern from spoilage and public health perspectives. Mainly in UK, Australia, USA and Canada the most common natural cheese used for processing is Cheddar cheese. Mozzarella cheese and Gruyère are used in Canada and the USA as well. Most often cheeses of Edam type such as Edam brick or Edam block are used for processing in the Czech Republic. Nevertheless, Swiss-type cheese, Moravian block or Primátor are used for processing minimally [7 – 12].

Other added materials can be divided into raw-milk and non-milk origin. As a raw-milk materials are used butter and cream. Theese raw materials can increase the FC in DM as well. Another raw material which is used may be cheese curd. Curd is added into the product because of increasing the non-fat solids content and contains intact proteins, mainly caseins with minimum proteolytic activity and minimal influence on the stability of the product and its consistency. Rework can be also used as a raw material because of its creamy consistency. Water is added into the product because of changing content of DM. Other raw materials as different flavour agents (meat ingredients, vegetables, spices, mushrooms etc.) can be used as well. The most important raw material are ES to obtain a smooth, fine and homogenous structure of the PC. The most commonly used are phosphate and citrate based ES [2, 5, 13, 15].

1.1.1 Natural cheese as the main raw material

Depending on the desired taste and consistency the main raw material of processed cheese is a natural cheese which can be made up of different species or be in different maturity stage [8, 12].

The monitored factors of natural cheese used as a raw material for the production of PC include mainly the pH, the calcium content, stage of maturity, FC and the amount of intact casein [7].

Edam, Edam block, Moravian block and Primator cheese are commonly used in the Czech Republic during PC manufacturing [12]. Especially in Anglo-American countries the most commonly type of cheese is Cheddar cheese [9].

For more cuttable product is suitable to use young or low-rippened cheese (usually 70 – 95 % of intact casein) because it results in a more stiff and gummy consistency of PC. Other advantages are high water binding capacity and lower production costs. The disadvantages are mainly empty flavour (low content of flavour compounds generated during prolonged maturation of natural cheese), worse meltability, possibility of excessive swelling and the formation of air bubbles due to high viscosity of melted material [9].

On the other hand it is preferable to spreadable products using of more ripened cheese (usually 60 – 75 % of intact casein). More easily meltable mixture with a fine texture can then be expected. The more matured natural cheese is, the more sensoric substances forms and thereby the final product aroma is more intensive [16 - 18]. When over rippened cheese is used, defects of consistency may occur. Defects can be responsible for a grain texture of the final product or the release of poorly emulsified fat and also give unstable consistency of the product [21]. The mixture of natural cheeses in different maturity stage are commonly used in practice [12].

In some cases the natural cheese frozened before the melting process is added. The frozen natural cheese increases hardness, decreases spreadability and lower in comparison with products in which the cheese was added unfrozen [9].

1.1.2 Butter

The amount and type of added fat can significantly influence the taste, structure and textural properties of PC. During the stirring and heating there is a decay of the natural fat globules, moreover melted fat forms particles of different diameters and then these are emulsified by caseinate. Emulsification stage plays an important role in shaping of the final structure. Increasing the degree of emulsification leads to a reduction of the diameter and increasing the amount of fat globules as result in a higher amount of bounds between the proteins. PC is than reflected in greater firmness but on the other hand lower stickiness.

The size of the fat globules may also affects the color of processed cheese. The smaller fat globules are, the more able to disperse the light and the color of PC is whiter [24]. Fat is added in the form of butter, cream or anhydrous milk fat.

According to Brickley et al. [30], absence of fats is problematic in the PC in order to color, texture, poor meltability and subsequent darkening and caking. Possible elimination of these properties was found as a reduction of DM content (density reduction of the protein matrix and better spreading), adjusting the pH (reaching the isoelectric point of casein). Casein molecules are aggregated to form a white color or by the addition of starter cultures (controlled acidification proces leads to decrease calcium content which decreases the density of protein network) [30].

1.1.3 Addition of skimmed milk powder or whey powder

Skimmed milk or whey powder is added in order to reduce price while producers attempt to reduce the amount of natural cheese. The addition of these two components leads to an increase of DM content in order to increase of lactose content of the melt. Increased content of lactose is reflected in break of compactness of protein matrix resulting in a lower hardness of PC. Undesirable phenomenon of using a higher amount of lactose is danger of non-enzymatic browning and formation of crystals both during production and during storage [9, 13, 27]. Kapoor and Metzger [9] recommends to lactose content in the final product does not exceed 7.48 % with regards to products of DM content of 44 % and 10.20 % for PC with a DM content of 60 % [9].

1.1.4 Addition of rework

Rework is a term used to describe the PC produced in industrial processes which was not further distributed. Rework may come from rejected batches due to unusual weight, packaging or quality defects. Since non-standard means the economic burden for manufacturers, rework is added to the melt and remelted. Content of added rework may range from 2 % – 15 %. Emulsification proces has already completed in rework contains ES. This is why addition of rework into the melt may be problematic and the properties of the final product can be influenced. Generally addition of rework leads to a reduction in spreadability and increase in hardness. The maximum added amount which does not influence the final product is 4 %. Also type of rework can affect the properties of the final product [9].

Rework is sometimes used to improve melting and functional properties of PC. Fresh rework has slightly dispersed (hydrated) protein structures and may be added in order stabilization of PC. There is a possible tendency to overcreaming under normal melting conditions of added amount from 2 % – 30 % depending on the degree of dispersion and emulsification [9].

1.1.5   Addition of calcium ions

Addition of calcium ions may influence the change in the consistency of PC namely an increase in hardness. The reason is large quantities of calcium ions involved in the formation of a protein matrix and intensively crosslink it. The disadvantage may be found in the reduction of natural cheese meltability [9, 30]. According to Mizuno and Lucey [33] calcium ions may be added only in limited amounts since excessive addition would cause excessive aggregation of the casein fractions (their hydrolysates) which would ultimately lead to the formation of unstable matrix [33].

1.2   Ingredients of non-dairy origin

Besides the PC, PC analogues can be also found on the market. Milk ingredients can be fully or partially replaced by raw materials of plant origin in these products. Non-milk origin proteins, vegetable oils, ES, flavouring agents, hydrocolloids and many other ingredients are used during processing. The advantage of these ingredients is the decreasing the production costs, because of expensive raw materials of animal origin and for this reason are replaced by lower cost plant raw-materials [2, 3, 16, 17].

1.2.1   Water

Water is added to PC in order to achieve the desired level of DM. Different addition of water can also influence the smoothness and spreadability of the final product and ultimately reduce the production costs. Hardness is decreased in order to addition of water while the meltability increases. The water addition influences release of calcium, hydration and dispersion of the proteins after addition of ES [31]. According to Lee et al. [31], decreasing the DM content reduces the size of fat globules. Reducing the DM content has a similar effect on mechanical properties as decreasing the temperature and pH of the raw material during the manufacture of PC. A disadvantage of PC with high water content is higher sensitivity to spoilage [31].

1.2.2 Hydrocoloids

In practice hydrocolloids are used both individually and in mixtures in concentrations up to 1 % (w/w). They are added as fillers, thickeners, emulsifiers and stabilizers of emulsions and foams. Some of them have the ability to form a gel (gelatin, starch, alginate, κ- and ι-carrageenan, agar, pectin, etc.). Hydrocolloids are widely used in various industries including the food, nevertheless they have their own place in the dairy industry [66, 67]. Selected hydrocolloids (carrageenan, cellulose, guar and locust gum) used Lu et al. [68], and found that the addition of the hydrocolloids hardness of PC increased. Macků et al. [69], describe hardness increase with increasing pectin concentration, κ- and ι-carrageenans Černíková et al. [70] and modified starch, low-esterificate pectine (alone or combined with lecithin) and locust gum as possible substitutes of emulsifying salts may be found in Černíková et al. [70].

Hydrocolloids may behave differently under different conditions (pH, concentration of electrolytes, production and storage temperature). Also interactions with other food components (proteins, polysaccharides, lipids, etc.) may ultimately affect their functional properties. Manufacturer task is therefore to choose the right kind and amount of a mixture of hydrocolloids in order to achieve the desired final product [65].

1.2.2.1 Alginates

Alginates is the general term for a salt of alginic acid. They can be extracted from seaweeds of the family *Phaeophyceae*. Alginates are products of *Azotobacter vinelandii* microorganisms or bacteria of the genus *Pseudomonas*. Alginates are capable of interacting with other electrostatically charged polymers (e.g. proteins) because they are polyelectrolytes. This property is used in the stabilization and enhancement of mechanical properties of the products and processed cheeses. If the interactions of alginates with caseins would be undesirable, mixing these components at higher pH is recommended. Most proteins then carries a negative charge and is difficult to binding alginates [79, 89].

1.2.2.2 Agar

The source of agars are red algae *Rhodophyceae* family. It is a linear polysaccharide, whose structural unit is D-β-galaktopysanose and 3,6-anhydro-α-L-galactopyranose alternately linked by glycosidic linkages (1 → 3) and (1 → 4). Basic neutral polysaccharide is agarose [65, 66].
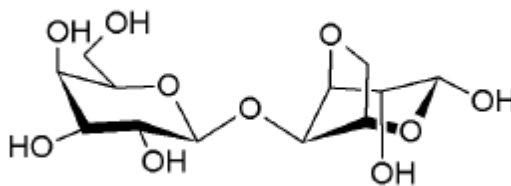
Fig. 1: Structural formula of agarose [66].

In the food industry it is used for its ability to bind water and formation of thermore-versible gel. Coupling of gels occurs via hydrogen bonds. Because gel formation is not conditioned by the presence of cations neither erratic cation content in dairy products did not affect gel formation, that is why alginates are used in food industry very often [65, 66].

1.2.2.3 Carrageenan

Carrageenans are natural linear polysaccharides extracted from red algae family *Rho-dophyceae.* The basic structure are a carabiose disaccharide, repeating sequence of β-D-galactopyranose and 3,6-anhydro-α-D-galactopyranosyl bonded by β-(1 → 4) linkage. Bond of α-(1 → 3) connects the individual disaccharides of carabiose [66, 70].

Carrageenans found application in many products for its stabilizing and gelling properties. They can be added in concentrations 0,01 − 0,30 % (w/w). In dairy products (shakes, ice cream, chocolate milk, pasteurized and sterilized creams) amount of 0.025 % (w/w) is used to stabilize and prevent the release of whey. For PC a part of ES can be replaced by carrageenan and the textural properties and feeling of fullness in the mouth is still mainta-ined. Carrageenans are not normally added in to the acidic products (soft cheese, yogurt). Low pH of products enhances electrostatic interactions between micelles of casein and carrageenan, resulting in unstable aggregates have a tendency to flocculate and separate itselves [72].

1.2.2.4 Pectin

Pectins are polydisperse polysaccharides of varying composition. They occur in the tissues of higher plants and are mainly obtained from citrus peels and apple pomace where their content is one of the highest [2, 66].
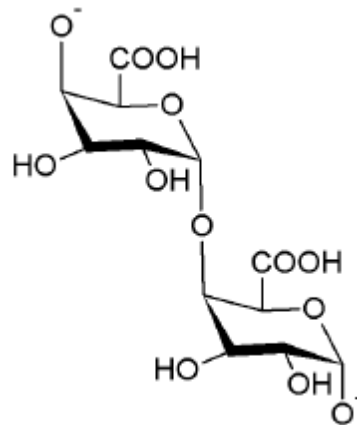
Fig. 2: Structural formula of pectin [66]

Pectins contributes to the stabilization of protein dispersion and at the same time are capable of gelation in dairy products. Pectins can be used for stabilisation of yoghurt milk, whey drinks and milk mixture with fruit juices. The effects of pectin are based in preventing sedimentation of casein micelles. Without the addition of stabilizer occurs to form a clear layer on the surface of the milk beverage which is entirely undesirable from a consumer point of view. In these dairy products high esterificated pectin is more effective stabilizer (it does not require the presence of calcium ions) in a concentration of min. 0.3 %. Mechanism of stabilization is based on interactions of pectin with casein. In yoghurt milk and yogurt which occurs as a result reducing the pH during fermentation by lactic acid bacteria to aggregation of the casein micelles and thereby destabilize these milk products with so called. Polymer stabilization takes place in yoghurt milk and youghurt because of aggregation of the casein micelles as a result decreased value of pH by low aktivity of lactic acid bacteria during fermentation. The polysaccharide molecules completely cover the present colloidal particles and thus the colluidal particles are stabilised. Thickening and gelling ability is mainly used to increase viscosity in yoghurts. The addition of pectin causes decrease of charge in the casein micelle and gel formation [72].

1.3 Processed cheese production

Continuous or batch processes can be used for PC production. During continous production a thin layer of the mixture is melted in stainless-steel pipes at a temperature of

130 – 140 °C for 2 – 3 seconds. Therefore, this cheese is so-called „*sterilised*" cheese while the batch process usually provides only pasteurization effect. Batch processig is more popular in the Czech Republic and is taking place in melting pots. This process involves:

- (I) the preparation of a mixture for melting,
- (II) determining the mixture of emulsifying salts,
- (III) the main melting process of the prepared mixture,
- (IV) packaging of the melt,
- (V) cooling,
- (VI) storage and
- (VII) expedition [8 – 10, 12].

A successful production is based on good quality and selection of natural cheeses. It is possible to use wide range of cheese in different stages of maturity. Criteria for selection of natural cheese and other raw materials depend on the DM content and the FC, flavor, texture, consistency and the pH of the final product. Natural cheese is peeld before the processing. This step is important for the melting process, proper „connection" of the added ingredients and entering of ES into the mixture [2, 8, 10, 12, 17].

Therefore, ES with polyvalent anions (especially phosphates) and monovalent alkali metals (especially Na) are commonly used during the production of PC. The amount of the added ES is usually 2 – 3 % of the weight of the raw product mixture [2, 3, 8, 10]. Hence, the maximum permitted amount of phosphate ES (expressed as P_2O_5) is 20 000 mg/kg for PC [19]. The maximum permitted amount of phosphoric acid, phosphates or its mixture is generally 40 000 mg according to the Regulation of the European Parliament and Council Regulation (EC) no. 1333/2008 [20]. Temperature and mechanical stirring in combination with the effect of emulsifying salts are the responsible factors for the physicochemical changes of the melt. This is why polyvalent anions affect the proteins, whose hydrophilic character is increased during the melting process and aglomeration of mixture starts to form. The bounded additional water increases the viscosity of the melt, leading to the creaming process.

Emulsifying salts can influence the pH and stability of the final product. The optimal pH range for spreadable processed cheese is 5.6 – 5.85. The added emulsifying salts influence the pH, structure and stability of the product [8 - 10, 17]. According to Guinee et al. [3] the emulsifying salts are „carriers" of functional properties of milk proteins. The basic functi-

on of emulsifying salts is the ability to cleave the calcium ions bounded into the natural cheese protein matrix and its exchange for sodium ions. Thus, less insoluble calcium paracaseinate is converted to the more soluble sodium paracasenate. Therefore, the key role of emulsifying salts is the modification of the environment in the melted mixture, so that proteins (casein fraction) can enforce their properties as true emulsifiers. On the one hand, overdose of specific emulsifying salts may lead to bitterness of the final product. The emulsifying salts will be discussed in next chapters [8, 10, 14, 17].
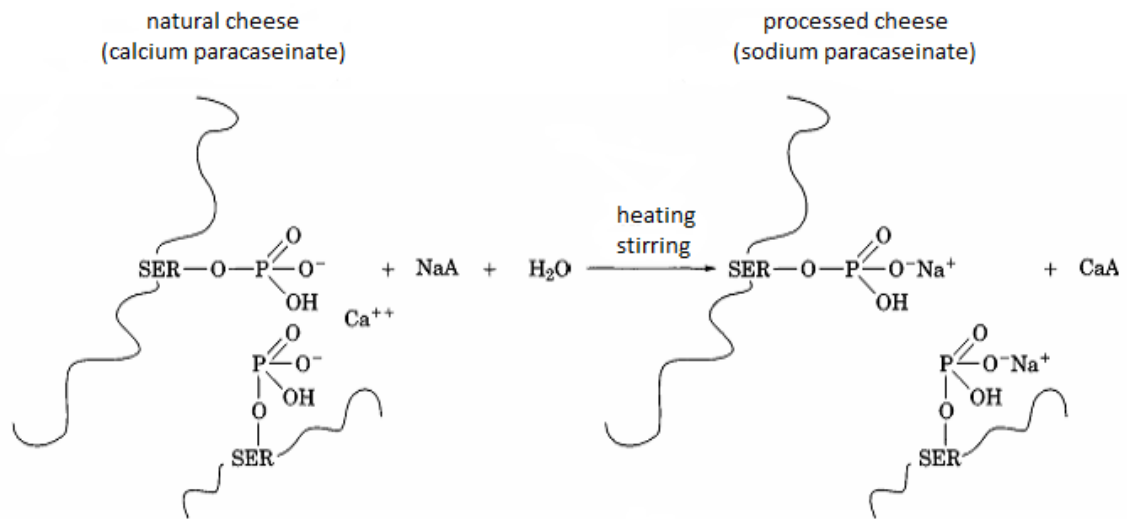
Fig. 3: Ion exchange scheme of the sodium ions exchange for calcium ions during melting of natural cheese [8, 14].

First of all, mixture of natural cheese is prepared and pulverized, after that conveyed to the melting pot, where the emulsifying salts and other ingredients are added. The melting pot is closed and partially vacuumed, because of air bubbles removal from the mixture. There is short time heated up to the melting temperature that usually ranges 90 - 100 °C. Moreover, heating may be performed through interspace or directly by injection of steam into the mixture. However, because steam is condensed inside the melting pot, this is why amount of water must be taken into account when it is added. The melting temperature is maintained for several seconds with constant stirring because of inactivation of pathogenic or sporulating microorganisms and also because of a stable final product with required sensory and textural properties (formation of a more viscous and creamier product). The more increased time of melting is, the more increased the firmness of the final product may

occur and that is why the product may be overcreamed [3, 10]. Thereafter, the melt is pac-ked, cooled and distributed further. Processed cheese is most commonly packaged in alu-minum foil. Nowadays, other packaging materials such as plastic cups, tubes, jars or metal cans can be used. Temperature is an important factor during packaging of processed chee-se. It should be around 70 °C because of microbial reasons and easier packaging, as well. The packed and cooled final products are stored at a temperature range of 4 - 8 °C [3, 8 - 14].

2 EFFECT OF EMULSIFYING SALTS ON THE CONSISTENCY OF PROCESSED CHEESE

According to the Regulation of the European Parliament and Council Regulation (EC) no. 1333/2008, ES are substances which convert proteins contained in cheese into a dispersed form, in order to achieve a homogenous distribution of the present fat and other substances. However, ES are often referred as emulsifiers, more particularly, they are emulsifying agents that help modifing the environment of the melt, so that the present proteins (the casein fraction) can „express" their properties as natural emulsifiers [8, 21, 22].

The most used ES are salts with polyvalent anions (phosphates, their polymers and citrates) and a monovalent alkali metals (especially sodium and potassium). The ES can be divided into three groups:

- (I) the monophosphates (orthophosphates),
- (II) polyphosphates and
- (III) citrates.

ES may be added either as one component ES mixture or as combinations of binary, ternary and even quaternary mixtures of ES in an amount of 3 g (the total quantity of used ES) per 100 g of mixture in the process of production of PC. Additionally, it is desirable to use mixtures of the right combination of ES in proportions which depend on the type of maturity and the structure of the used natural cheese for the formation of a homogeneous mass. The composition and amount of ES significantly affect the final texture of PC. The higher the concentration of added ES is, the firmer structure of PC can be expected [2, 8, 10, 12, 24, 26, 27].

The basic ability of ES is the removal of calcium ions from the protein matrix, peptization, hydratation of proteins, thus increasing their solubility and promoting the ability of proteins to swell. ES also help to emulsify and stabilize the present fat within the formed matrix. Nevertheless, heating of the mixture without the addition of ES would cause the destruction of the membrane on the surface of the fat globules and subsequent coalescence of fat globules could occur. Due to high temperature and low pH, aggregation and contraction of the caseins can cause separation of fat, water and proteins. Generally, in order to form a homogeneous mass, the addition of ES is necessary [5, 8, 10, 21, 22].

2.1 Phosphate-based emulsifying salts

Phosphates are compounds derived from orthophosphoric acid (H_3PO_4) containing anion $(PO_4)^{3-}$. Orthophosphates are salts containing only one phosphorus atom, therefore, they contain one group of $(PO_4)^{3-}$. Condensation of orthophosphates formed salts with two groups of $(PO_4)^{3-}$, so-called pyrophosphates. The compounds formed by condensation of orthophosphate containing more than two phosphorus atoms in the molecule are called polyphosphates or condensed phosphates. These polyphosphates may be subdivided into three groups, on the compounds forming:

- (I) linear chain of polyphosphates,
- (II) closed cycle polyphosphates called metaphosphates and
- (III) compounds having a three-dimensional structure called ultraphosphates [8 – 10].

Phosphate ES affect especially the ion-exchange between the calcium and monovalent ions, adjusting the pH of the environment, its stabilization (buffer capacity), and fat emulsification. The basic ability of ES is cleavage of the calcium ions bounded on the protein matrix of the natural cheese and sodium ions can be exchanged. This allows the conversion of insoluble calcium salts of caseins (calcium paracaseinate) on soluble sodium salts (sodium paracaseinate) which helps the hydration process and fat emulsification. This attribute is crucial because it determines the actual process of PC production. Moreover, due to ion exchange it is possible to obtain a homogeneous structure of the final product during the production of PC [8, 2, 3, 23, 27].

The ability of the ion exchange groups of phosphates is depending on the number of phosphorus atoms in the molecule. Therefore, the affinity for cations increases with the increasing number of phosphorus in the compound (polyphosphates > pyrophosphates > orthophosphates). The ability of ion exchange is further influenced by the pH and temperature. The ion exchange ability is increasing with the increasing temperature and with the increasing pH level, as well [8, 12, 13, 21, 28 – 30].

Using of phosphates influence the pH of the environment and stabilize the pH, because of buffer capacity. As mentioned before, the optimal pH of spreadable PC ranges 5.6 – 5.8. In particular, phosphates have different pH, therefore, it is necessary to choose a suitable combination in order to obtain optimal pH and desirable textural properties of the final product [8, 10, 17, 21, 31]. Selected phosphates at a concentration of 3 % w/w and pH of 1

% of aqueous solutions are shown in Table 1 [2, 8]. Buffering capacity of phosphate ES is dependant on their chain length. The longer the chain, the buffering capacity descreases. The most intense stabilization ability of pH possess orthophosphates. Thus, with the increasing length of polyphosphate chain, the pH of the solution and PC decrease [8, 10, 21, 31].

Table 1: The phosphates used as emulsyfying salts in the manufacture of processed cheese [2, 8].

Group	Substance	Formula	pH in a 1 % aqueous solution	E - codes
Orthophosphates	Sodium dihydrogen phosphate	NaH_2PO_4	4.5	E 339
	Disodium phosphate	Na_2HPO_4	9.1	
	Sodium phosphate	Na_3PO_4	11.9	
Pyrophosphates	Disodium dihydrogen pyrophosphate	$Na_2H_2P_2O_7$	4.1	E 450
	Sodium pyrophosphate	$Na_4P_2O_7$	10.2	
Triposphates	Sodium triphosphate	$Na_5P_3O_{10}$	9.7	E 451
Polyphosphates	Sodium polyphosphate (Graham's salt)	$(NaPO_3)_n$	6.6	E 452

Another important property of phosphates is to improve the emulsification of fat and proteins. Great impact on the emulsification has a the pH of the environment. With its increase are removed more calcium ions from the casein chains. Consequently, the chains a delayed and the emulsifying capacity of the present caseins is improved. Furthermore, the size of fat globules is reduced and their specific surface area is increased. Further reducing of the fat globules size, may lead to the protein chains again approach each other and begin to interact together. Poor emulsification causes the excretion of fat globules from PC. On the contrary, good emulsification causes the production of soft or tougher PC [16].

In addition, another property of phosphate ES is the ability to affect the formation of a gel, which depends on the type of phosphate [26, 28, 32]. The low molecular weight phosphates (pyrophosphates and tripolyphosphates) promote the crosslinking of the gel matrix more than linear polyphosphates. High molecular weight phosphates are considered as inhibitors of gel formation. During gel formation a complex of casein and calcium phosphates developes and that is why the repulsive forces between casein chains are reduced [5, 8, 27, 33]. During production and storage of the final product, linear condensed phosphate are hydrolyzed to individual orthophosphates. The most intensive reaction is the decomposition of polyphosphates to pyrophosphates. The reaction rate is slowing down during the decomposition of polyphosphates into orthophosphates. The extent of hydrolysis is dependent on the time and the temperature of treatment of the product, further to the duration and temperature of storage, the pH and the length of phosphorus chain. The higher indicated values are, the hydrolysis of phosphate increases [21, 27, 34]. Hydrolysis influences changes in the buffer capacity of ES and decreasing the affinity of the ES to calcium ions. However, the decomposition of phosphates may also support undesirable formation of crystals, which are formed in the presence of pyrophosphates and orthophosphates. Temperature and mechanical stress are responsible for increase of the water binding capacity in proteins, thereby the viscosity of the entire system increases [8, 21].

2.2 Citrate-based emulsifying salts

Citrate salts are salts derived from tricarboxylic citric acid. Sodium citrate was the first ES used for the manufacture of PC. The most common ES based on citrates is trisodium citrate ($C_6H_5Na_3O_7$). Monosodium citrate and disodium citrate are used very rarely because of acidification of the final product is high, resulting in the formation of an unstable emulsion which easily releases water. Citrates are used in combination with other salts during production of PC especially polyphosphates [8 – 10, 21, 26]. Citrates affinity for calcium ions as compared with phosphates is low, on the other hand, is higher for sodium ions. Citrates have low ability to increase hydratation of the proteins and fat emulsification. Moreover, citrate are also considered as substances which do not participate in crosslinking of the protein matrix. In comparison with phosphate, bactericidal or bacteriostatic activity was not prooved. In addition, potassium citrates are not applied during the production of PC because they can create a bitterness of the final product [2, 3, 8, 9, 13].

Table 2 shows citrates which are used as ES and the value of pH in a 1 % (w/w) aqueous solutions [8, 14].

Table 2: The citrates used as emulsyfying salts in the manufacture of processed cheese [8, 14].

Group	Substance	Formula	pH in a 1 % aqueous solution
Citrates	Sodium citrate	$NaH_2C_6H_5O_7$	3.7
	Disodium citrate	$Na_2HC_6H_5O_7$	4.5
	Trisodium citrate	$Na_3C_6H_5O_7$	5.6

3   FACTORS AFFECTING THE CONSISTENCY OF PROCESSED CHEESE

Besides flavor consistency is one of the main features of PC [2, 59]. PC are found in a range of consistencies from delicate, through the well spreadable to liquid consistency. The consistency of PC is determined by many different factors (e.g. use of ES, composition and degree of maturity of natural cheese, use of additives, processing conditions and interaction between other factors). Generally, these factors can be divided into three groups:

- (I) the composition of the raw material mixture,
- (II) processing parameters during the production of PC (especially the speed of agitation, holding time, the temperature reached during the melting process and the rate of cooling),
- (III) the storage temperature and period [74].

3.1   Composition of the raw material mixture

The composition of the raw material mixture includes mainly the type of the natural cheese and its degree of maturity, DM content, FC in DM, pH value, proportion of calcium ions in the melt, amount of the cream and lactose content. Moreover, a key factor affecting the consistency of the processed cheese is amount and the ratio of the applied ES [8, 12].

The basic raw material for the production of processed cheese is the selected natural cheese. Natural cheese has a significant impact on the consistency of the final product, namely its calcium and casein content and pH value. These properties are „modified" during ripening of the natural cheese. Therefore, it is important to consider the type of natural cheese, its degree of maturity, its composition, pH value and the desired properties of the final product for the cheese processing. Unripened raw material (70 – 95 % of intact casein) generally results a stiffer and more rubber consistency [2, 59].

Young cheeses are mainly used for the production of sliced cheese. The disadvantage of young cheeses is the emptiness in flavor because of low content of sensory active compounds, worse melting ability, danger of excessive swelling and the possibility of formation of air bubbles due to high viscosity of the melted mixture. On the other hand, using of ripened cheese (60 – 75 % of intact casein) is achieved softer and more spreadable consistency, because the mixture of mature cheeses is easier to melt and the final product has

usually fuller and stronger flavor. During the ripening of natural cheese, proteolysis occurs. Proteolysis is one of the most complex and important microbiological and biochemical processes that takes place during ripening of most natural cheeses. Hydrolysis of caseins to peptides and free amino acids is in progress during this process. The casein proteolysis is „realised" by enzymes, including a residual enzymatic activity of the raw milk, clotting agent and an exogenous enzyme system of fermented- and non-fermented lactic acid bacteria (LAB). The content of intact casein in the casein matrix can influence the strength and the fragility of cheeses. When low-fermented raw material with a low degree of proteolysis is used, the final product has more rubber and stiffer consistency. When ripened cheese with a high degree of proteolysis is used, the final product has a soft and spreadable consistency [2, 17, 60, 61].

Another important factor affecting the consistency of PC is the relationship between DM content and fat in DM. The stiffer consistency of the product can be achieved at constant fat in DM content by an increase in the DM content. On the other hand, increasing the FC at a constant DM content can be achieved more spreadable consistency [2, 55, 60]. The consistency of PC can be influenced by the content of calcium ions which come from natural cheese into the product. Additionally, Kapoor et al. [59] investigated the effect of increasing the content of calcium ions in Cheddar-processed cheese. The latter authors found that the high content of calcium ions in the raw material results in a more firm final product [59]. Moreover, another important factor influencing the consistency of the product is the pH. This pH value is mainly affected by type and concentrations of the used ES and the pH of natural cheese. The „older" (more ripened) natural cheese is used during the manufacture of PC, the higher pH product is formed. The added ES are not only to promote emulsification properties of proteins but they can adjust the pH of the melt to the optimum pH value 23]. According to Marchesseau et al. [17], the optimal pH of the PC in the final product is 5.6 − 6.1. The closer the pH of the processed cheese to the isoelectric point of casein (pH ~ 4.6) is, thus, a more rigid consistency of the final product forms. According to Lee and Klostermeyer [31], a low pH (4.8 − 5.2) of the final product can be formed using ES like sodium citrate, sodium phosphate and sodium metaphosphate. Consequently, a dry, hard and crumbly PC can be expected. At high pH (> 6.0) there is a decrease in the electrostatic interactions and increase in the negative charge of the proteins which can cause their repulsion. These weakened gel is characterised by a very soft consistency [17, 61]. A significant role in forming the texture of PC play ES (their amount and composition). In gene-

ral, with the increasing content of ES of phosphate and citrate-type (about 0 – 3 % w/w), the rigidity of the final products increases [2, 26].

3.2 Processing parameters

3.2.1 Processing and cooling of the melt

Melting temperature, intensity and duration of the stirred hot melt and the cooling rate are factors contributing to the consistency of the PC. The most important parameter is the melting temperature. According to Guinee et al. [3], the increasing value of the melting temperature reduces the strength of the formed gel [2, 3, 10, 12]. However, excessive endurance at temperatures of 75 – 90 °C can lead to overcreaming of the mixture, resulting in a hard and pudding consistency of the final product, which can be characterised by oiling off (release of fat from the matrix). The consistency of the PC can be influenced by the pattern of the melting process. An increasing duration and intensity of stirring of the melt has great influence on the consistency. This factor can determine the size of the fat globules. Increasing length and intensity of mixing improves fat emulsification, resulting in the increase in stiffness of the PC. Moreover, the cooling rate is a factor affecting the consistency of the product. Generally, slow cooling leads to an increase in the stiffness of the final product, whereas rapid cooling can provide a less rigid final product [2, 26, 64].

3.2.2 Storage conditions

According to the Decree no. 397/2016 Coll., the consistency of PC is also influenced by storage period and transport. An increase in stiffness takes place during storage period of PC [21, 27, 34]. Polyphosphates are gradually hydrolysed into simple phosphates. Hence, the reduce of the number of monomers in a linear-chain polyphosphates results in a decrease in affinity for calcium ions. Therefore this leads to a gradual release of these ions from the ES and subsequent integration into the protein matrix. [2, 22, 60]. Higher storage temperature (25 – 30 °C) could lead to increased stiffness of the PC [26, 27]. According to Awad et al. [27], it has been shown that at higher storage temperatures (20 ± 2 °C) was observed an intensive formation of darker color of the product than the products stored at lower temperatures (7 ± 2 °C) [2, 27]. Long-term storage (3 – 4 months) of PC can be a problem with the microbiological properties and the shelf life of these products. Microbiological changes are influenced by several factors, namely the type of natural cheese, DM

content, pH and heating temperature. Shelf-life of stiff PC is higher than for softer produ-cts, because the spreadable consistency products generally contain higher amount of water [61, 64].

3.2.3   Agitation speed

Noronha et al. [76] focused on the effect of different agitation speeds (100 – 1500 rpm) at a constant holding time (~2 min) at 80 °C on the consistency of PC block imitations. Their results showed that with the increasing speed of agitation, the firmness of the final product, its cohesiveness and the storage modulus G` increased while the size of the fat globules decreased [76].

4 PROPERTIES OF WHITE-BRINED CHEESE

4.1 White-brined cheeses

According to the Ministry of Agriculture Decree no. 397/2016 Coll. cheese is defined as a milk product produced by precipitation of milk proteins from milk by the action of rennet or other suitable coagulating agents, separating of the whey and after that, fermentation or maturation process begins [3].

White cheese is a traditional food in the regions of the Balkans and the Middle East. Early reports indicate that the art of cheese-making was brought originally from Asia to Europe through the Middle East. Until late in the nineteenth century, cheese was exclusively prepared at home or produced on farms. Nowadays, most cheeses are produced in dairy factories, but some varieties are still produced on a small scale under domestic conditions. Therefore, almost all varieties of cheeses which are produced in the Middle East region, are white cheeses, most of which are matured in brine [35].

White brined cheeses (WBC) are cheeses ripened and preserved in brine, made from curds that are not subjected to any heat treatment, like Feta and similar cheese varieties (Akawi, Jadel, Telemea, Domiati, Halloumi, Brinza). Their flavour is slightly acidic and salty, that sometimes can become rancid and piquant. Their colour is pure white, when they are made from ewe's and goat's milk. The cheese mass has no rind, no gas holes or other openings, except for small mechanical openings and its texture is smooth and rather soft but always sliceable. The size and shape of the white, rindless cheese group varies but the typical form is square, 7 x 7 x 7 cm^3 with an approximate weight of 1 kg. [36, 37].

Moreover, Akawi cheese is a very popular type of cheese, produced on a large scale in many countries in the Middle East region, notably, Lebanon, Syria and Cyprus. Akawi cheese is used extensively in the preparation of many dishes and foods that contain cheese as a major ingredient, such as traditional Middle Eastern confectionery products (sweets). The cheese is desalted by soaking in water for several hours or overnight, before it is utilized in the preparation of sweets. Akawi is a white, pickled cheese made in many countries from locally available milk source [35].

4.2 Production of White-brined (Akawi) cheese

Traditionally, Akawi cheese is produced from pasteurized bovine or ovine milk (or a mixture of them). For Akawi cheese-making, milk is pasteurized (at 60 °C for 30 min or 72 °C for 15 s), cooled to about 35 °C and then starter culture (mostly *Lactococcus lactis*, *Lactococcus lactis* subsp. *cremoris, Lactobacillus casei*) is added (1.5 %, w/w). After 1 hour, rennet is added to coagulate milk within an hour. Therefore, the curd is cut, the whey is drained, and curd-pieces are wrapped in cheese cloths in small portions (150 to 250 g), pressed for about 1 hour, and brined in approximately 10 % (w/w) brine solution at 4 °C. In general, Akawi cheese contains about 51.0 % moisture, 21.6 % fat, 22.5 % protein, and 5 % ash. Moreover, the storage of Akawi cheese in brine solution is a critical step for maintaining quality and safety during storage [38, 54].

In retail marketing, the cheese is kept in the brine until the day it is displayed for the consumer. Several blocks are taken out, and are displayed in a refrigerated cabinet for the day's sale [35].

Akawi cheese is a salty white cheese that has few particular characteristics, and hence, is rarely found to be faulty. Sometimes, it may be too salty, but this is not unexpected and consequently cannot be considered as a defect, since it is normal practice to reduce the salt content by soaking the cheese in water before consumption [35].

Because of the high salt content 5.42 – 5.58 % (w/w) and health concerns, Middle Eastern people avoid consumption of Akawi cheese because of a positive correlation has been found between high sodium (Na) intake and hypertension [38], osteoporosis [39], kidney stones [40], and cardiovascular diseases [41].

4.3 Ripening of white-brined cheese

Maturation of cheeses is a complex of physical, chemical and mainly biochemical changes that result in the formation of sensory active substances and typical characteristics of cheese (color, consistency, flavor and aroma). During maturation, the cheese becomes softer which is caused by the hydrolysis of casein micelles by proteolysis, the change of water binding in curd and pH changes. Biochemical changes during the maturation process can be divided into two phases. The primary phase includes the metabolism of lactose, lactate and citrate, lipolysis and proteolysis. Secondary phase playing an important role in forming taste, include metabolism of fatty acids and amino acids [42, 50].

Many significant changes in the constituents and properties of WBC take place during its maturation, which contribute very much to the development of its physicochemical and organoleptic properties. These changes are affected by many different factors, such as the quality of milk, the pasteurization, the lactic acid starters, the temperature of the ripening room [35].

4.3.1 Lactose, lactate and citrate metabolism

A key process during the ripening is the metabolism of lactose to lactate using LAB, known as acid cultures (e.g. *Streptococcus thermophilus*, *Lactobacillus helveticus*). The extent of acidification of the curd affects its initial structure. Ripening is influenced by pH, which affects the texture directly that can influence the solubility of casein. Cheeses with a higher pH are softer than cheeses with lower pH. Also, the enzyme activity is influenced by pH. Most of the lactose present in milk is excluded into the whey during cheese production, however, part of it still remains in the curd. Therefore, it is important to complete the fermentation of lactose which prevents inappropriate development of secondary microflora. The residual lactose is metabolised by LAB at the beginning of maturation in order to produce lactate [55, 56, 57]. Lactose which remains unfermented by LAB is probably metabolized by non starter-LAB (e.g. *Lactobacillus casei*, *Lactobacillus delbruecki*, *Lactobacillus plantarum* or *Lactobacillus rhamnosus*) [55].

4.3.2 Lipolysis

Lipolysis is the metabolism of triacylglycerols to fatty acids which contribute significantly to the taste formation of natural cheese. The resulting fatty acids are precursors for the formation of volatile flavours. During maturation, lipolysis is mediated by the activity of the -starter and non-starter cultures and the residual lactic lipase (plasmin, lipoprotein lipase) which is thermolabile but whose activity is reducing by heating more than 50 °C. Enzyme activity is influenced by the surface of fatty ball membrane which is made up of proteins and phosphoproteins.

If the membrane is damaged by mechanical damage such as homogenization or foaming, an increase in the surface tension of the fat ball occurs and lipolytic enzymes can readily penetrate through the membrane into the fatty ball and due to its high lipolytic activity produce unwanted taste of the final product [13, 52, 55].

The occurrence of foreign odors is dependent on the renneting of milk. Nutrition for dairy cows often reflects on the taste of milk in which the foreign odors can forms. Some enzymes can also produce an unfavorable taste of the product such as lipase-induced rancidity [55].

Free fatty acids (FFA) and free amino acids (FAA) are considered to be very important compounds for cheese flavour. Very important, from this point of view, is not only their total concentration in the cheese, but also the quantities of the different free amino acids and free fatty acids, and the ratio between them [35].

The rennet used in brined cheeses traditionally containes pregastric lipases, and in developed technologies, lipase is added. Lipolysis is therefore, one of the characteristics of brined cheeses. The mono- and diglyceride content of the cheese increases on storage with the formation of variable FFA. However, the volatile fatty acids of brined cheeses contain a high percentage of acetic acid, originating from microbial fermentations. The high content of these acids affects the flavour of the final product. [35].

Lipolysis can be expressed as FFA content. Brined cheese contains in general up to $4 - 6$ g FFA per kg of cheese, including acetic acid. It has to be mentioned that very high values (up to 10 g/kg) have been reported for brined cheese made with artisanal rennet [44, 45]. The main organic acids throughout of brined cheese ripening are lactic, citric and acetic acids, depending on the starter used. Among them acetic acid is a characteristic element of brined cheese flavour and increases significantly during ripening. In mature brined cheese it ranges from 0.4 to 1.5 g/kg [36, 45 - 48].

4.3.3 Proteolysis

Proteolysis is the most important primary biochemical reaction that occurs in cheese during ripening. Proteolysis contributes to softening the cheese structure during maturation by hydrolysis of the casein matrix and by reducing water activity. It has a direct effect on cheese flavor due to the production of short peptides and amino acids that are precursors of secondary biochemical reactions [55].

Cheese proteolysis, primarily of casein components, is considered to result from several proteinase and peptidase activities. The principal proteolytic agents in cheese are proteinases of rennet, indigenous milk proteinases (especially plasmin), and proteinases and peptidases from starter-, non-starter and secondary starter bacteria. Other original proteinases in

milk can be produced from leukocytes of the somatic cells. Somatic cells contain many proteinases including cathepsins type B, D, G, H, L and elastase. Proteolysis in cheese is affected by the pH of the curd, salt in moisture content of the cheese and ripening time [42, 43, 49, 50].

Primary proteolysis in cheese may be defined as those changes in α_{s1}- and β-caseins and-peptides therefrom, which can be detected by PAGE [51]. Primary proteolysis of cheese proteins is mainly the result of the action of indigenous proteinases (i.e., plasmin, and perhaps cathepsin D and other somatic cell proteinases) and residual coagulant. However, proteinases from starter- and non-starter microorganisms are also active in the degradation of cheese proteins and peptides [43, 52].

The proteinases of rennet are mainly responsible for the initial proteolysis of the caseins in cheese during ripening due to the residual rennet activity retained in the curd. These enzymes modify the texture of cheese by slowly degrading α_{s1}- and β-caseins, which are responsible for forming the framework of the cheese matrix, and produce sapid compounds or, more usually, their precursors. LAB are weakly proteolytic but possess a very comprehensive proteinase/peptidase system capable of hydrolysing casein derived peptides to small peptides and amino acids. *Lactococcus spp.*, *Lactobacillus spp.* and *Streptococcus thermophilus* when used as starters have extensive activity in cheese, particularly in the production of short peptides and free amino acids [43, 49, 52]. The levels of intact protein and of protein breakdown products in cheese at various stages of ripening have been used as indicators of maturation, because the extent of degradation is associated with the development of flavour and texture in most cheese varieties [43, 51, 53].

The products produced by the catabolism of amino acids are aldehydes, ketones, alcohols, aromatics, hydrocarbons, amines, ammonia and sulfur compounds. LAB are the major agents of amino acid catabolism but propionic bacteria also have a high ability to transform the branched chain of amino acids (isoleucine, leucine) into a branched chain of fatty acid (isovaleric acid) [15].

II. ANALYSIS

5   THE AIM OF THE WORK

The aim of this Master's thesis was to study the influence of the maturity stage of White-brined cheese on the consistency of processed cheese with different composition of emulsifying salts.

The aim of the theoritical part of the Master's thesis was:

- (I) Processing of a literary research focusing on the characteristics of processed cheese and description of emulsifying salts, particularly phosphate and citrate emulsifying salts,
- (II) A description of the maturing process of White-brined cheese.

The aim of the experimental part of the Master's thesis was:

- (I) To produce processed cheese model samples with four types of ternary mixtures of emulsifying salts,
- (II) Investigating the influence of ternary mixtures of emulsifying salts on the textural properties of processed cheeses and the influence of the maturity stage of natural cheese (used as the main raw material) on the textural properties of processed cheeses after basic chemical analysis and textural profile analyzes.

6 MATERIALS AND METHODS

6.1 Experiment description

The experimental part of the thesis included the production of model samples of PC using 4 types of ES. Trisodium citrate was used as ES (TSC; $NaH_2C_6H_5O_7$), disodium hydrogenphosphate (DSP; Na_2HPO_4), tetrasodium diphosphate (TSPP; $Na_2H_2P_2O_7$) and sodium salt of polyphosphate with a mean chain length of n $\approx$ 20 [P20; $(NaPO_3)_n$]. From these types of ES, 4 combinations of ternary mixtures were created: (I) DSP:TSPP:P20, (II) DSP:TSPP:TSC, (III) DSP:TSC:P20 and (IV) TSC:TSPP:P20. Each combination of ternary mixtures of ES was applied in varying percentages in 12 variants (100:0:0, 50:50 0, 100 0, 40:40:20, 40:20:40, 20:40: 40, 50:0:50, 0:50:50, 40:0:60, 20:20:60, 0:40:60, 0:0:100).

For the pH adjustment in the production of model samples of PC a basic solution of sodium hydroxide (NaOH) with concentration c = 1 mol/l or acidic hydrochloric acid (HCl) with concentration c = 1 mol/l was used to obtain the optimal pH at the interval of 5.60 – 5.80. The calculated amount of solutions was subtracted from the total amount of water addition in order to maintain the desired DM content of the final product. These solutions were added to the melt during the melting process at 85 °C. All types of model samples were produced in duplicate. The samples were stored at 6 ± 2 ° C and further analysed on the 2nd, 9th, 30th and 60th day after the production day (day 0).

A basic chemical analysis was carried out which included the measurement of the pH of the developed samples and the determination of the DM content of the PC as well. Moreover, the textural profile analysis aimed at monitoring the textural characteristics of the samples, namely hardness, cohesiveness and relative adhesiveness.

6.2 Production of model samples of processed cheeses

The experimental part of the current thesis began with the production of the model samples of PC [with a DM content of 40 ± 2 % (w/w) and a FC in DM of 50 ± 2 % (w/w)].

The basic raw materials used for PC production are listed below:

- (I) Akawi White-brined cheese [(DM content 48 % (w/w), FC in DM 38 % (w/w), maturity 2, 4, 8, 16 and 24 weeks (at 6 ± 2 °C), producer Mlékárna Olešnice, RMD, Olešnice, Czech Republic)],

- (II) Butter [(DM content 85 % (w/w), FC in DM 82 % (w/w), manufacturer Sachsenmilch Leppersdorf GmbH, Germany)],
- (III) Mixture of ternary mixtures of ES: disodium hydrogenphosphate (DSP), tetrasodium diphosphate (TSPP), sodium salt of polyphosphate (P20) (producer Fosfa a.s., Břeclav, Czech Republic), trisodium citrate (TSC) (producer Merck, Dormstadt, Germany),
- (IV) Water.

Prior to the production of the PC desalination of the raw material (Akawi WBC) was realised. The proces of desalination was performed by soaking the natural cheese in plastic barrels (volume of 50 l) containing potable water. The water was changed every 3 hours. The desalination process was chosen due to a decrease the initial value of the salt content in the cheese used as raw material to approx. 2.5 % (data not shown). The ratio of water to natural cheese in the barrel was 8:1. The production of model PC was carried out using the Vorwerk Thermomix TM 31 – 1 equipment (Vorwerk & Co., GmbH, Wuppertal, Germany) at a melting temperature of 90 ± 1 °C with a duration of 1 minute. Adjustants of pH (NaOH or HCl) were added at 85 °C to reach values of pH of the samples in the range of 5.60 – 5.80 (optimal pH of spreadable PC). The total melting time was 9 ± 2 minutes. Upon the completion of the melting process the resultant melt was transfered into polypropylene cups (diameter 52 mm, height 50 mm) which were then sealed with aluminum caps. These samples were further stored at 6 ± 2 °C until the analyses were performed.

6.3 Basic chemical analysis

The basic chemical analysis included pH measurement and determination of DM content of the produced samples. The pH values were measured using a pH-meter with a pH glass electrode (pH Spear, Eutech Instruments, Oakton, Malaysia). In the experiment the pH-meter was applied to each cup (respectively PC sample) in a total of three times in randomly selected locations. The determination of DM content was performed according to ČSN EN ISO 5534 by drying the test sample at a temperature of 102 ± 2 °C up to constant weight loss [77].

6.4   Textural profile analysis

For the texture profile analysis, the TA.XT Plus device (Stable Micro Systems Ltd., Godalming, United Kingdom) was used. The texture analysis was performed by penetrating the sample using a 20 mm diameter cylindrical probe to a depth of 10 mm. The speed of this probe was 2 mm/s.
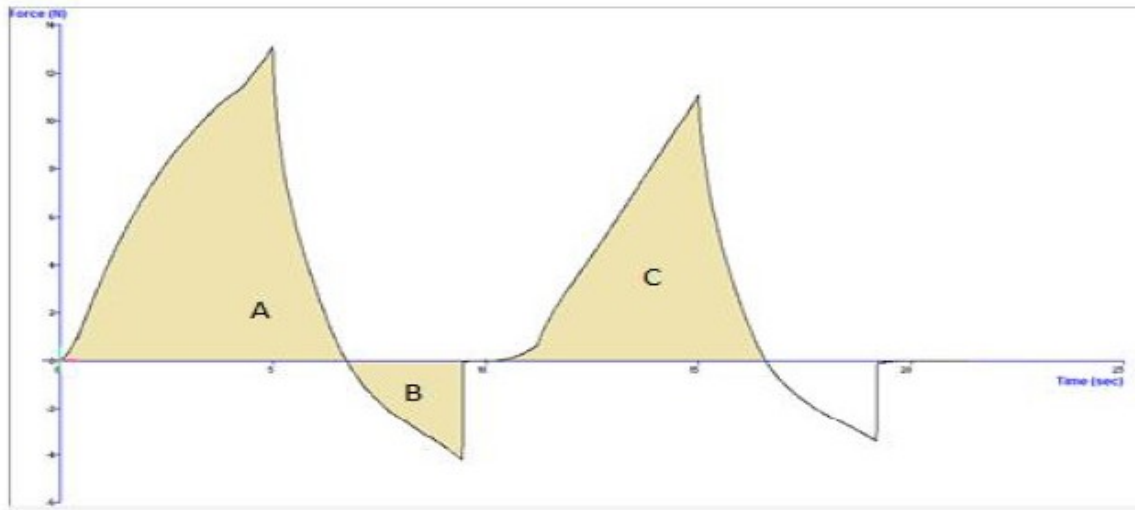
Fig. 4: *Stress curve describing the dependence of deformation force (N) on time (s), adjusted according to [78]*

The values of hardness, cohesiveness and relative adhesiveness of the samples were monitored. Figure 4 shows the curves describing the dependence of the force (N) applied to the deformation on the time (s) from which the values mentioned above were determined.

As hardness (N) of the sample is defined the force required to achieve product deformation. The higher the maximum peak curve A, the harder the product is, and the more force it takes to deform the examined PC [26, 31, 79].

The relative adhesiveness is due to the work required to overcome the attractive forces between the surface of the PC and the surface of the probe. The relative adhesiveness was determined as the ratio of the absolute value of the negative peak B to the area of the positive peak A [26, 31, 79].

Cohesiveness is defined as the strength of the inner bonds that „make up" the food. The evaluation is given by the ratio C:A [26, 31, 79].

6.5 Statistical evaluation of data

The measured and detected data were subjected to statistical analysis using the Kruskal-Wallis test and the Wilcoxon test. All statistical evaluations were performed at a 5 % significance level. The Unistat ver. 5.5 software was used for the calculations (Unistat, London, United Kingdom).

7 RESULTS AND DISCUSSION

7.1 Basic chemical analysis

7.1.1 Determination of dry-matter content

The values of DM content of the processed cheese samples ranged from 40.16 – 41.11 % (w/w). This range corresponded to the target value of the DM content of the examined samples which was 40 % (w/w). Comparable values of DM content are necessary to ensure the standardity of the analysed samples because the DM content significantly influences the textural properties of PC [31, 80, 81].

7.1.2 Determination of pH values

Processed cheese pH values significantly affect the consistency of PC and are dependent on the composition, type and amount of the applied ES [17, 31]. In evaluating the results of pH, it was found that, when separate ES were used, the highest pH values were obtained in the samples prepared with disodium hydrogenphosphate and tetrasodium diphosphate (DSP and TSPP). The pH values ranged from 5.51 to 6.27. On the contrary, the lowest pH values were in the range of 4.74 – 5.42 which were monitored by the application of the sodium salt of polyphosphate (P20) as sole ingredient. The pH values for PC with individually applied trisodium citrate (TSC) behave similarly to DSP and TSPP salts. The optimum pH for spreadable PC is usually in the range of 5.6 – 6.1. These values are reported by Marchesseau et al. [17], Lu et al. [82], Buňka et al. [12]. Our goal was to achieve optimal pH in the range of 5.6 – 5.8. Generally, with the increase in the content of the used ES, the hardness of the final products increases [2, 28]. Lee and Klostermeyer [31] reported that dry, hard and friable PC resulted from the formation of a low pH (in the range of 4.8 – 5.2) using trisodium citrate, sodium phosphate and sodium metaphosphate. Marchesseau et al. [17] stated, at a lower pH than the optimum, caseins are approaching their isoelectric point (pH ~ 4,6). There is a reduction in the negative charge, thereby enhancing aggregation and weakening the emulsification of the present fat in the PC. In addition, protein molecules are not sufficiently hydrated, causing the production of a dry and grained PC [17]. At high pH (> 6.0), a decrease in the electrostatic interactions (increase in the negative charge of proteins) can occur, which causes them to repel. This weakened gel is then mani-

fested especially in a very soft consistency [17, 31] which can be confirmed also by our results.

In the following figures (Figures 5 – 24) are depicted the results of pH values development using 2, 4, 8, 16 and 24 week natural cheese, depending on the composition of the ternary mixtures of ES and the storage duration (60 days in total).

The pH values for the teranry mixture of ES composed of DSP:TSC:P20 mixture are shown in Figure 5 - 9.
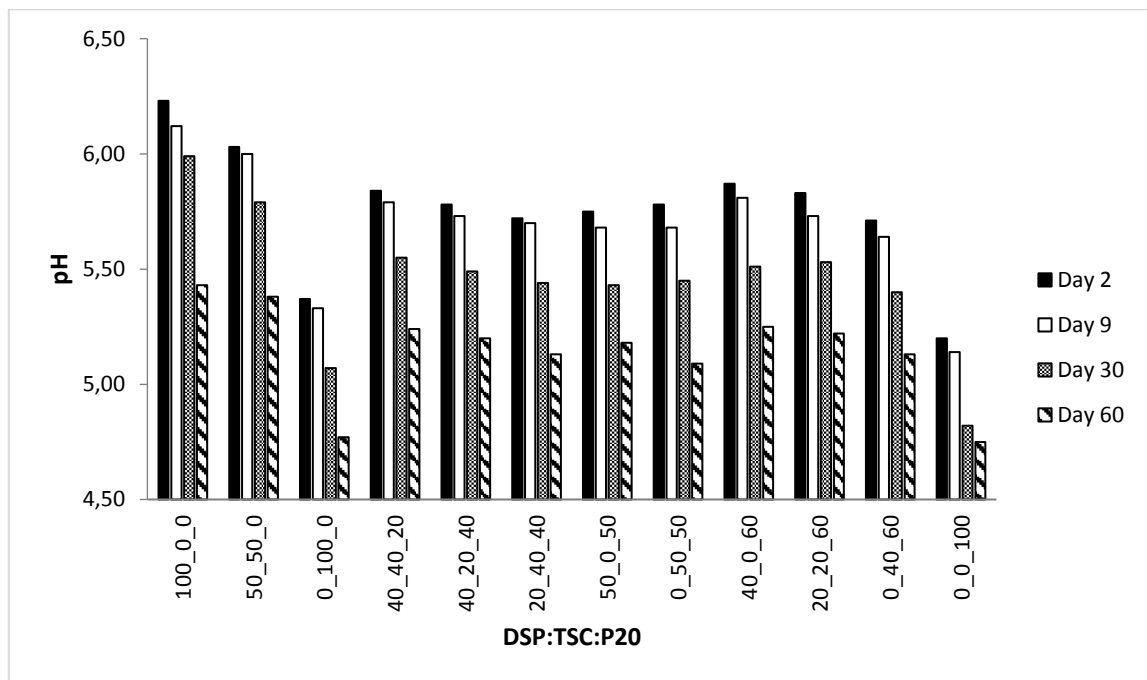
Fig. 5: pH of model samples of processed cheeses made from a two-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage duration (60 days)
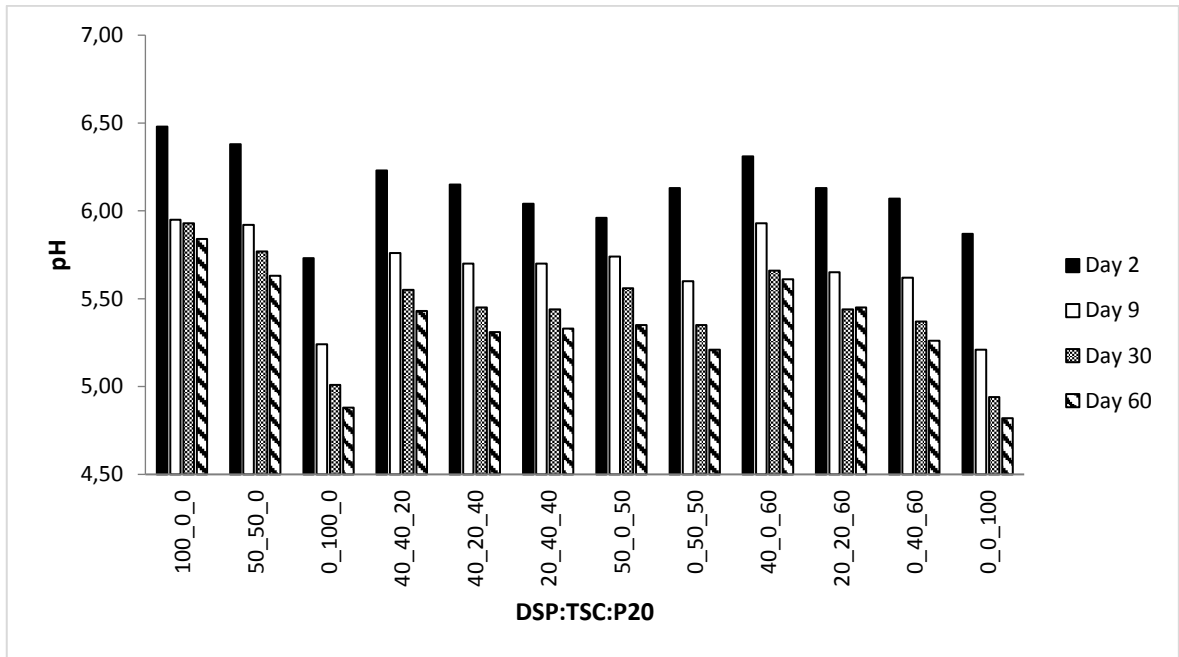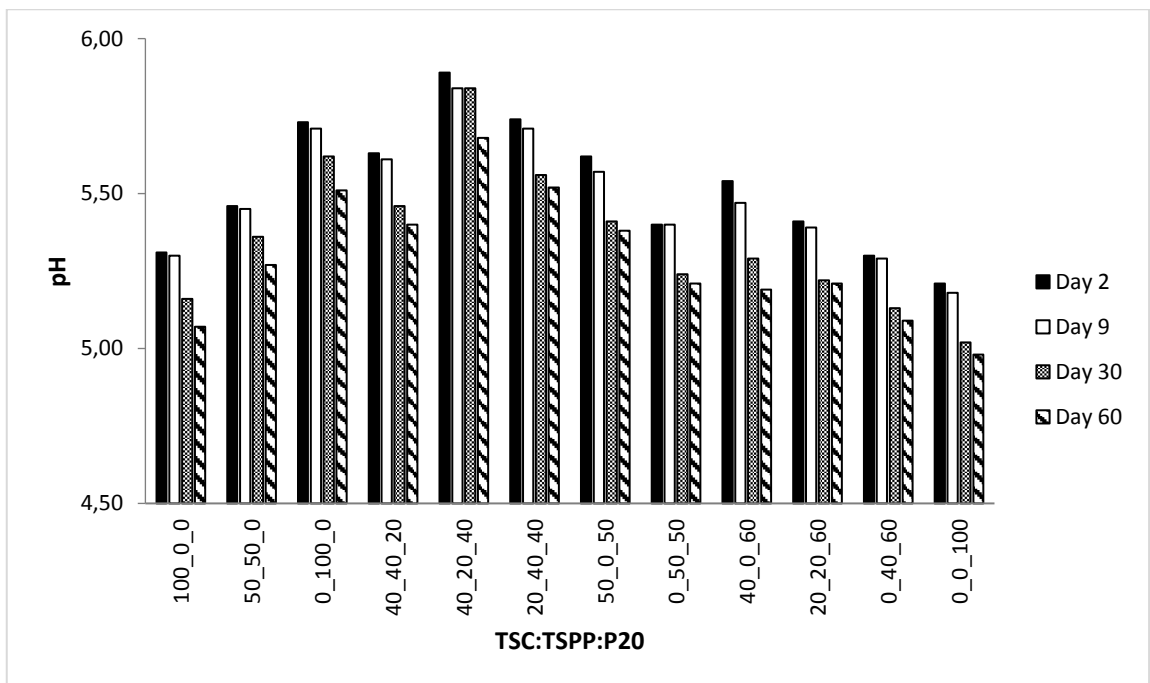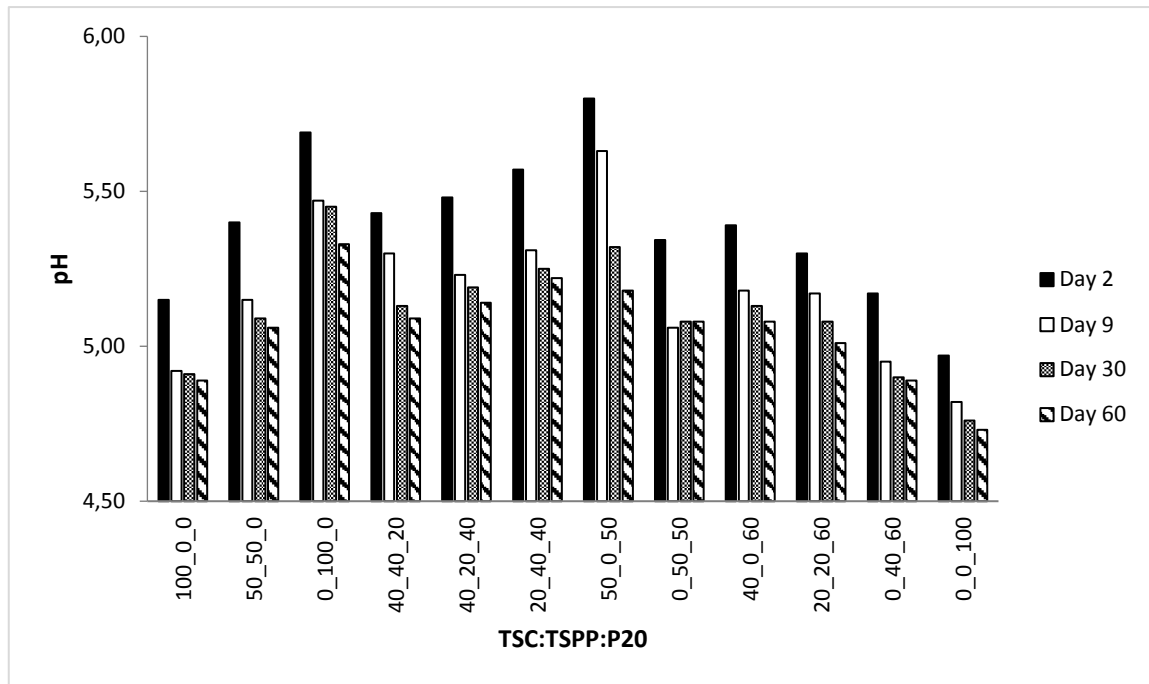
Fig. 6: *pH of model samples of processed cheeses made from a four-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage duration (60 days)*
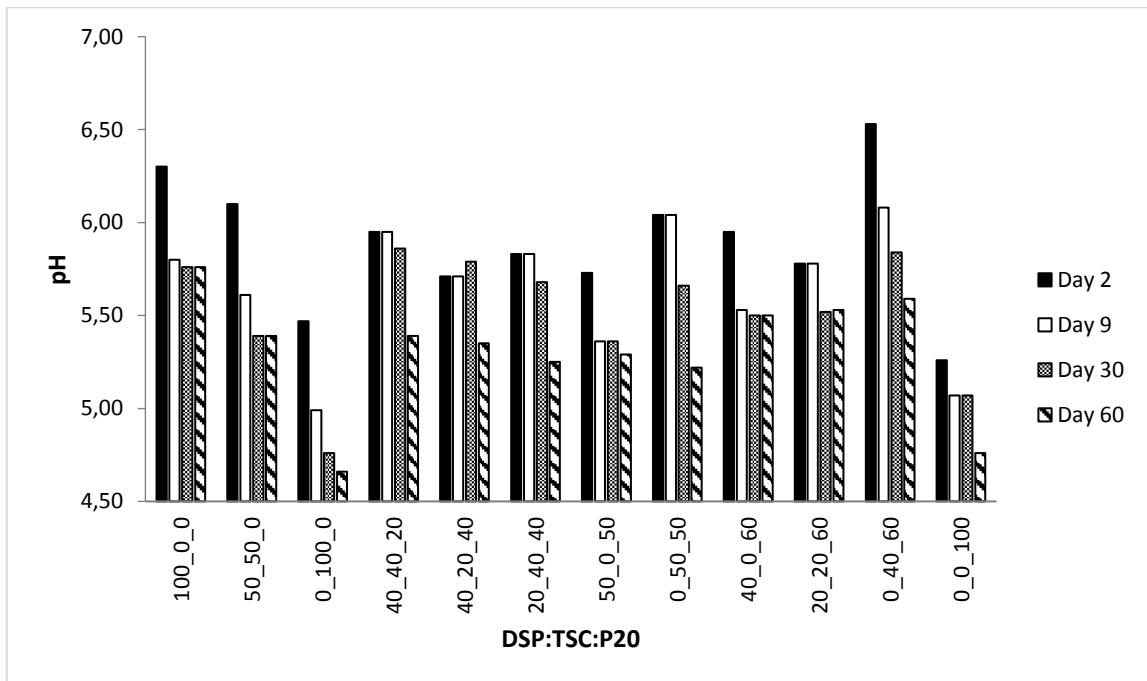
Fig. 7: *pH of model samples of processed cheeses made from a eight-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage duration (60 days)*
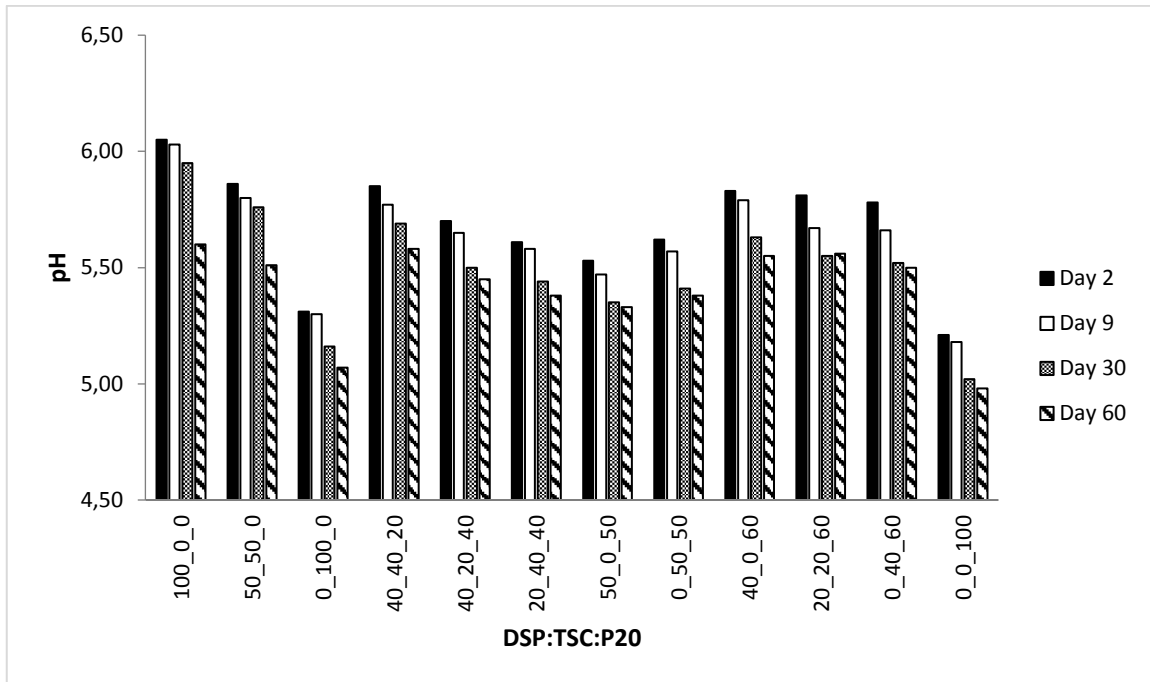
Fig. 8: pH of model samples of processed cheeses made from a sixteen-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage duration (60 days)
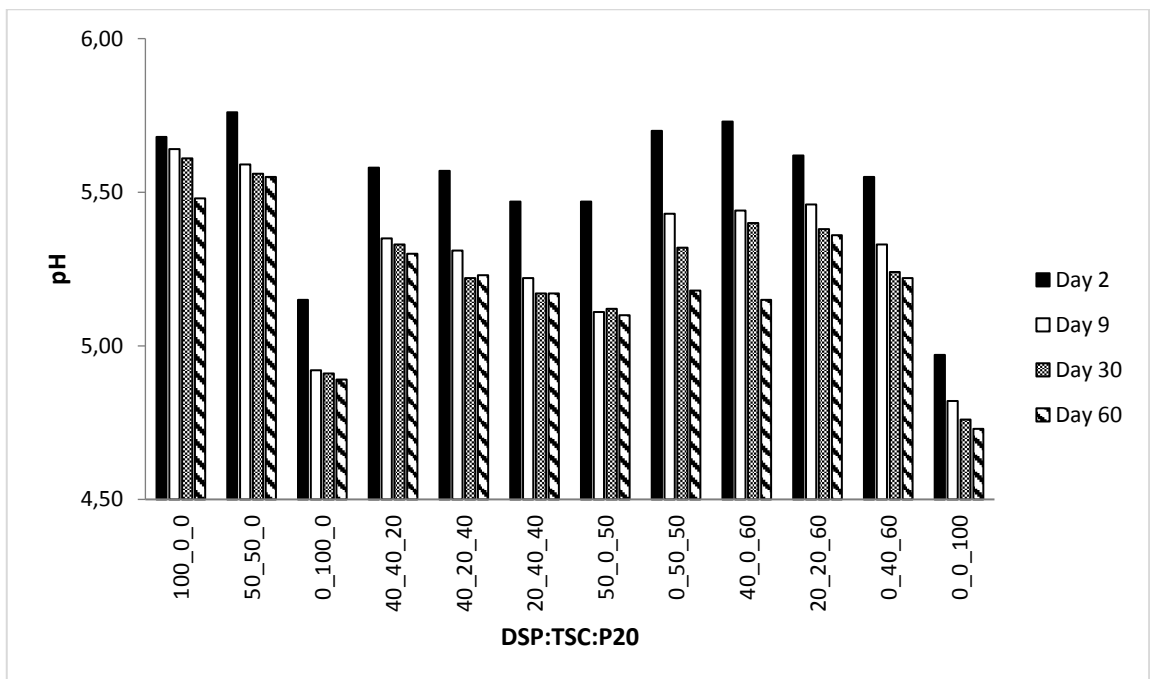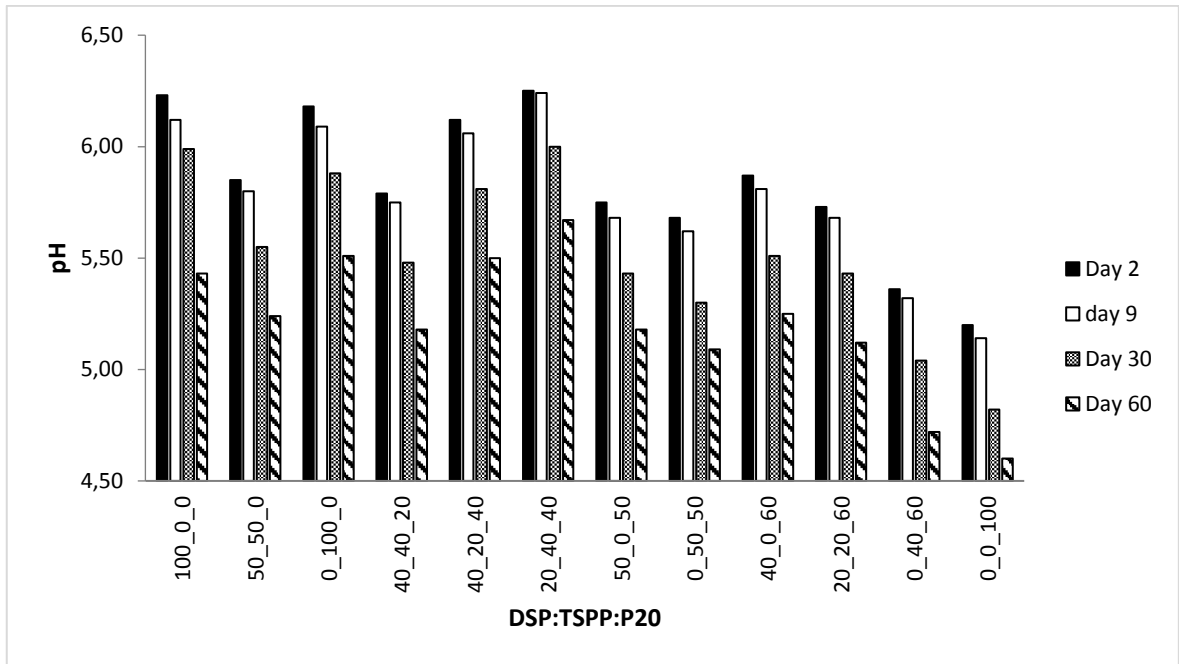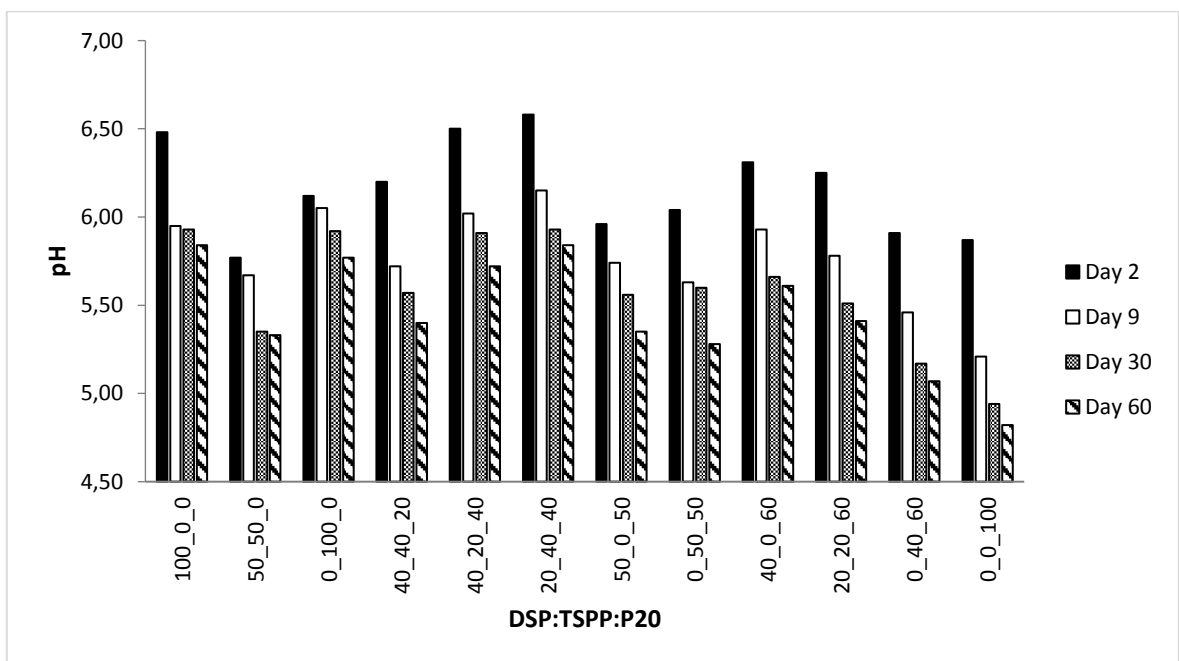

Fig. 9: pH of model samples of processed cheeses made from a twenty four-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage duration (60 days)
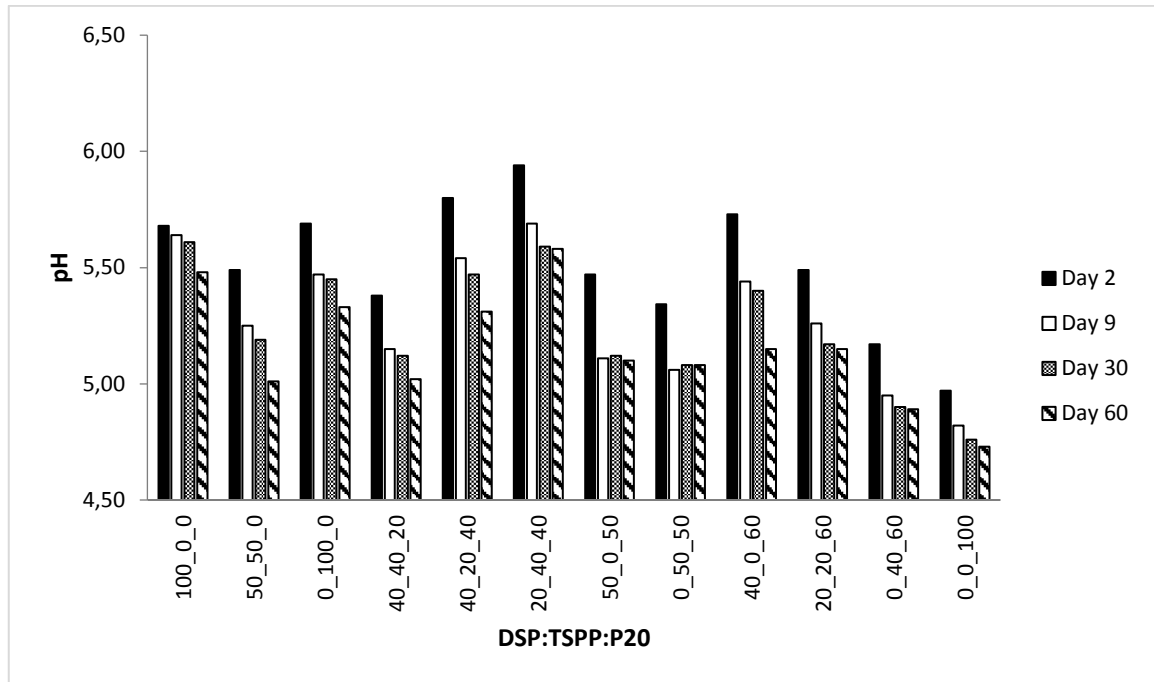
Based on the pH results of model samples of PC using a combination of ternary mixture DSP:TSC:P20 and natural cheese using as raw material at varying maturity stages, a slight decrease in pH was observed during the storage period of all model samples of PC. This reduction in pH values is explained by Awad et al. [83], namely, by the hydrolysis of phosphate ES during the storage period [29, 83]. Howeever, decrease in pH values were observed not only for condensed phosphates but also for monophosphates in which no further hydrolysis is expected. Changing of the pH value in this situation can be explained by a possible change in the form of binding of presented ES. Polyphosphate hydrolysis takes place during the production of PC. The extent of these decomposition processes depends on the length of the polyphosphate chain, the length of the processing time, the melting point and the storage time, respectively. Hydrolysis increases with the increasing values of the parameters mentioned above [21, 27, 34]. The present polyphosphates are progressively hydrolysed into monophosphates. The effect of hydrolysis of phosphates is to change the buffering capacity of ES and to decrease the affinity of ES for calcium ions, resulting in a decrease in the pH values. This phenomenon was described Muslow et al. [23], Shirashoji et al. [26] and Mizuno and Lucey [33].

Using the combination of ternary mixture DSP:TSC:P20, the lowest pH values were observed when applying TSC salt as sole to the 8-week raw material; which was 4.66 ± 0.02. On the other hand, the maximum pH of the 8-week raw material which was 6.53 ± 0.02 was observed in the ternary mixture at a ratio of 0:40:60. This phenomenon can be explained by the functional properties of ES. As a result of the addition of P20, the negative charge intensity increases in the casein chains, creating a looser matrix and thus, creating a dull consistency of the product with high pH values [23, 33, 84].

According to the figures presented below (Figure 10 – 14), during the storage period of the model samples using a ternary mixture of DSP:TSPP:TSC, was observed a slight decrease in the pH values. It was also observed that decreasing the TSPP content in the ternary mixtures resulted in a decrease in the pH of the end-products. At 100 % TSPP in the mixture, maximum pH values were observed in the range of $5.33 - 6.52$. This phenomenon can be explained by the functional properties of TSPP. Mizuno and Lucey [33] indicated, that TSPP promotes gel formation of the present proteins and consequently reduces the negative charge of caseins [33, 85]. It can be said therefore, that with the increasing ratio of TSPP in the mixture, the negative charge and thus the pH of the final product increases.
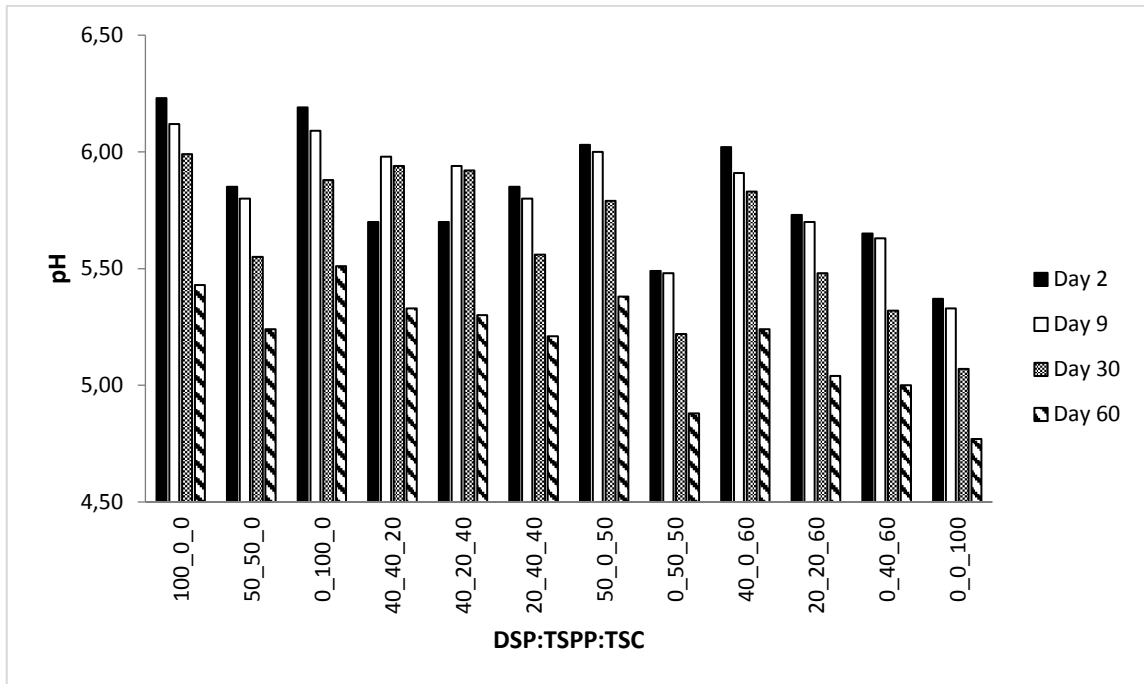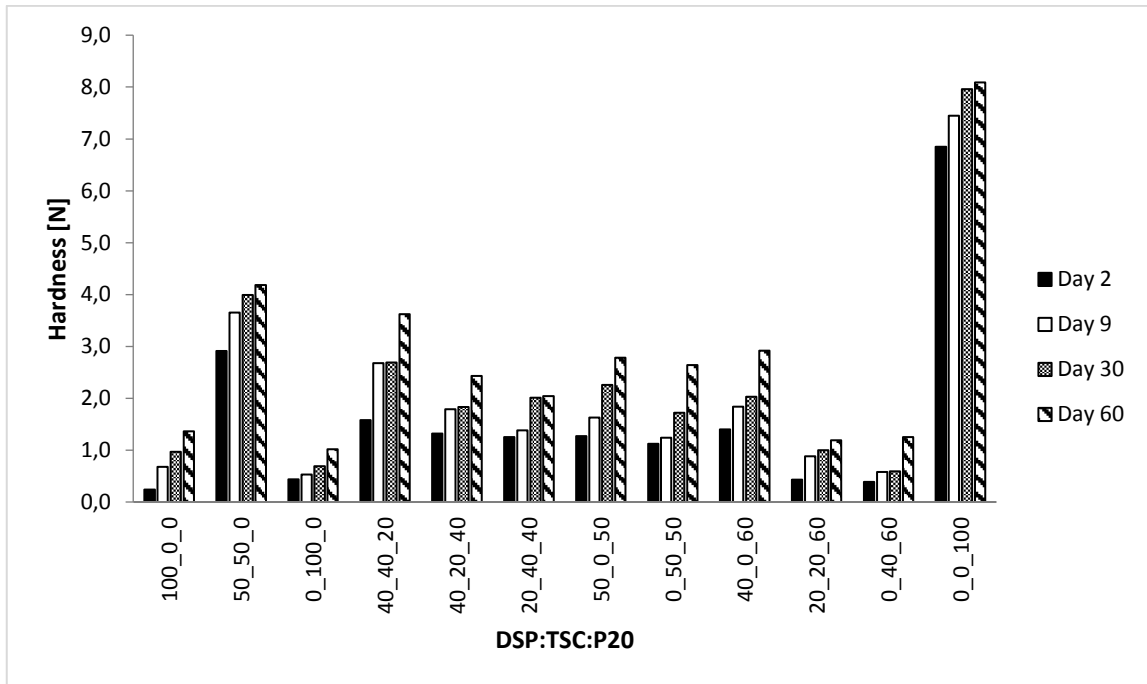
Fig. 10: *pH of model samples of processed cheeses made from a two-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage duration (60 days)*
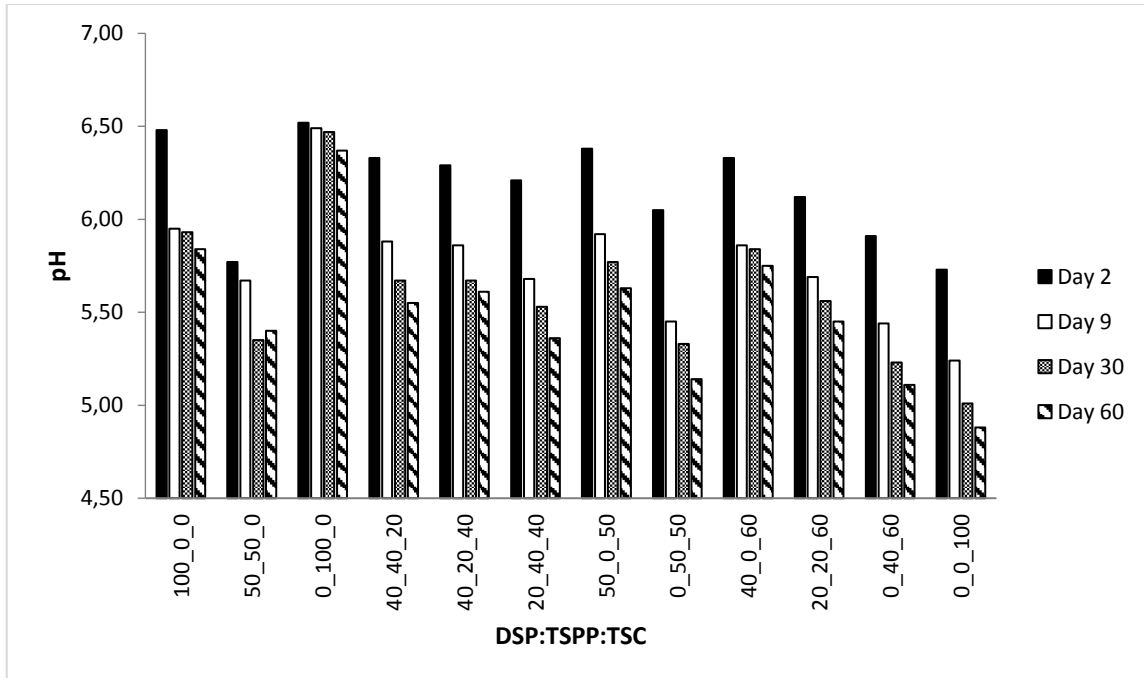
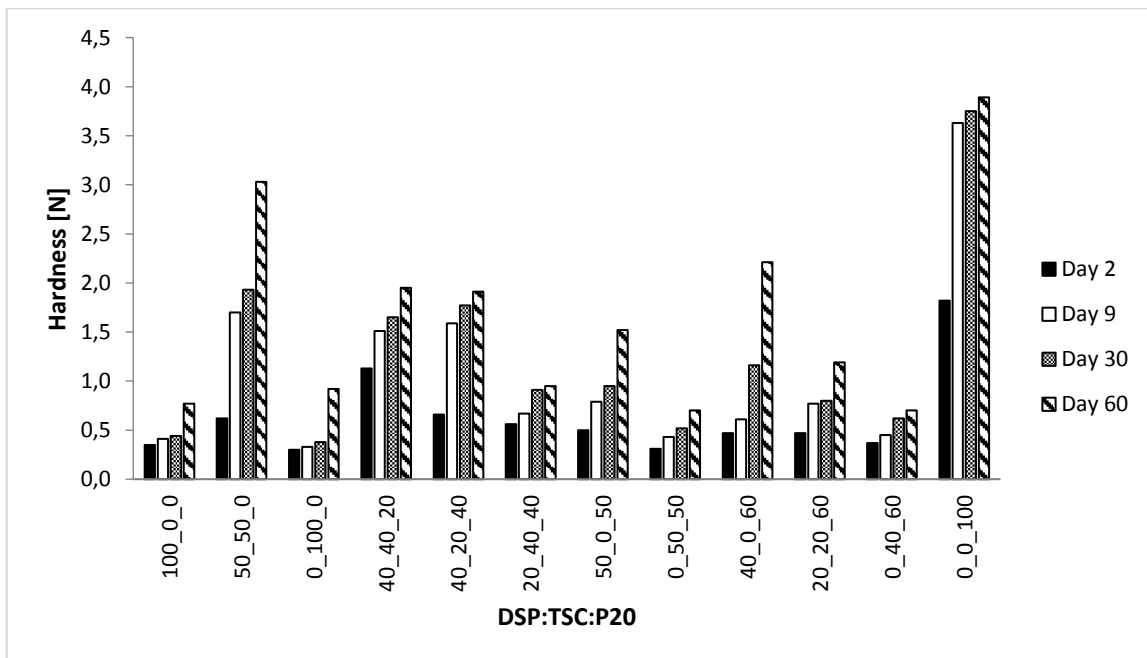*Fig. 11:* *pH of model samples of processed cheeses made from a four-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage duration (60 days)*
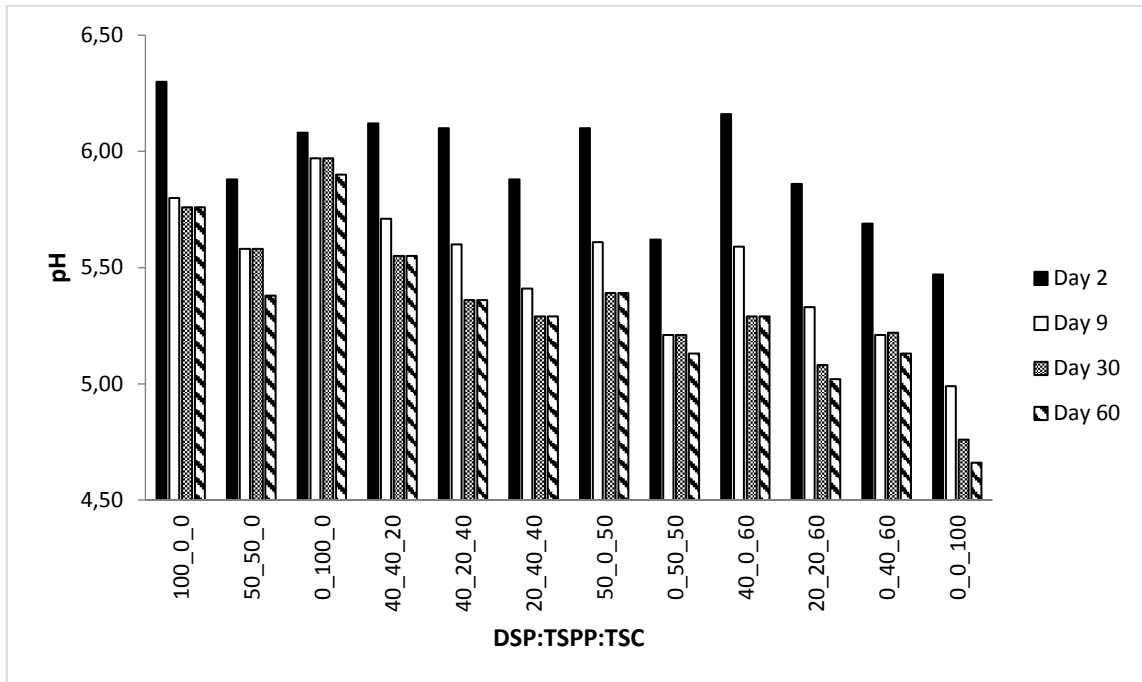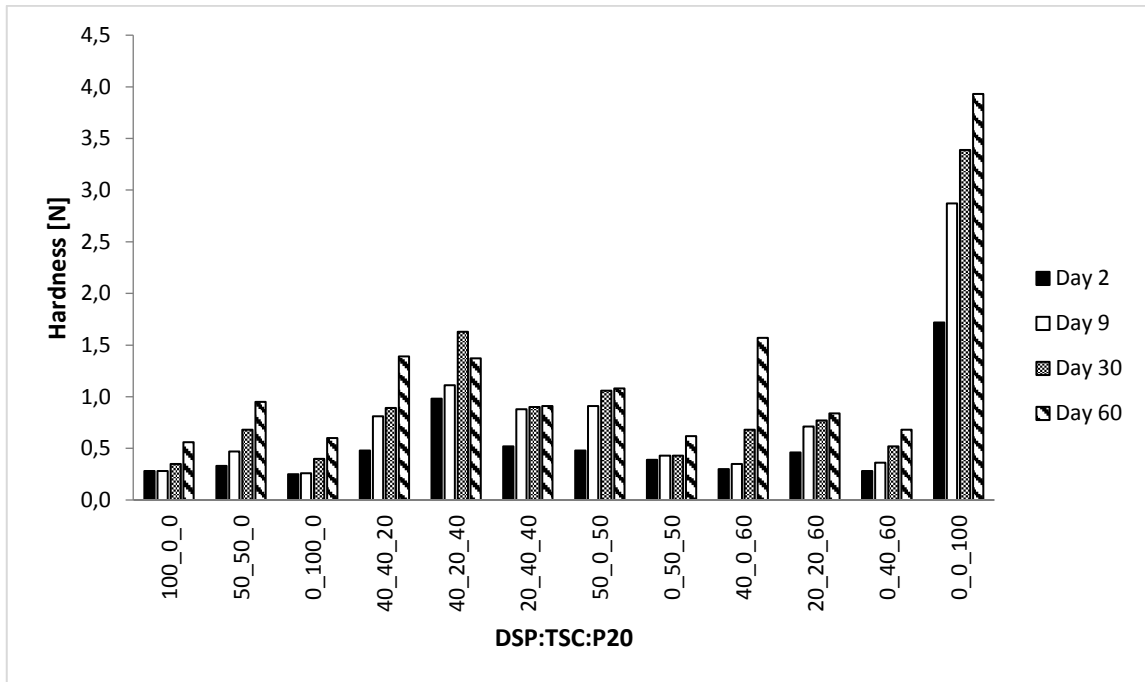
Fig. 12: *pH of model samples of processed cheeses made from a eight-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage duration (60 days)*
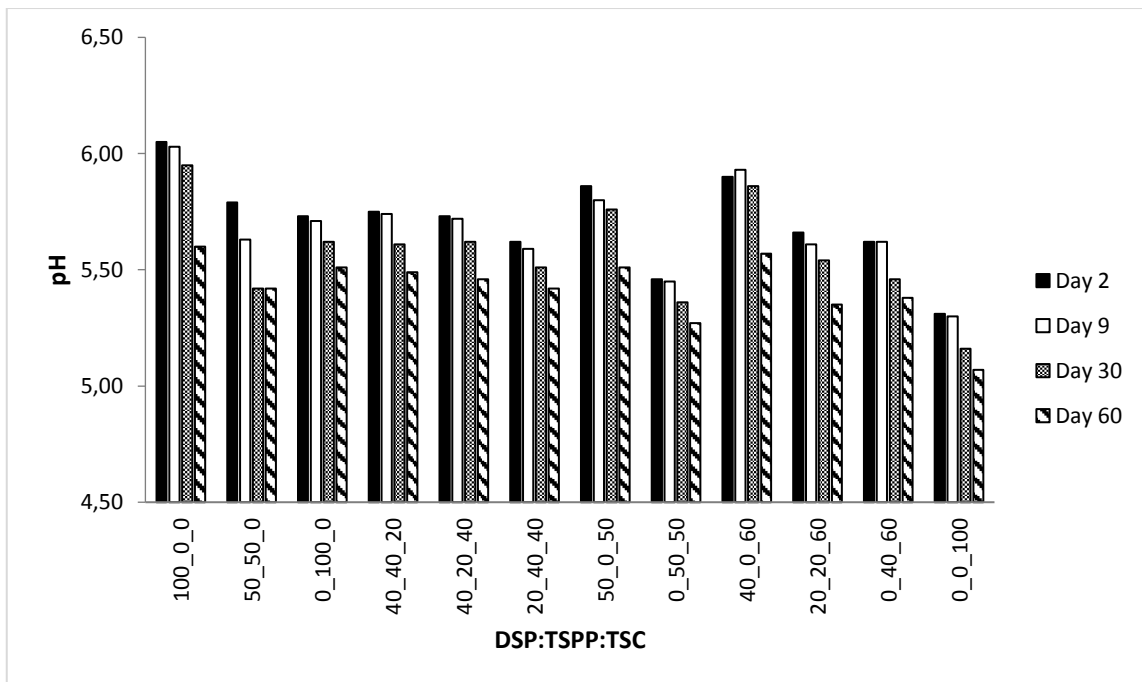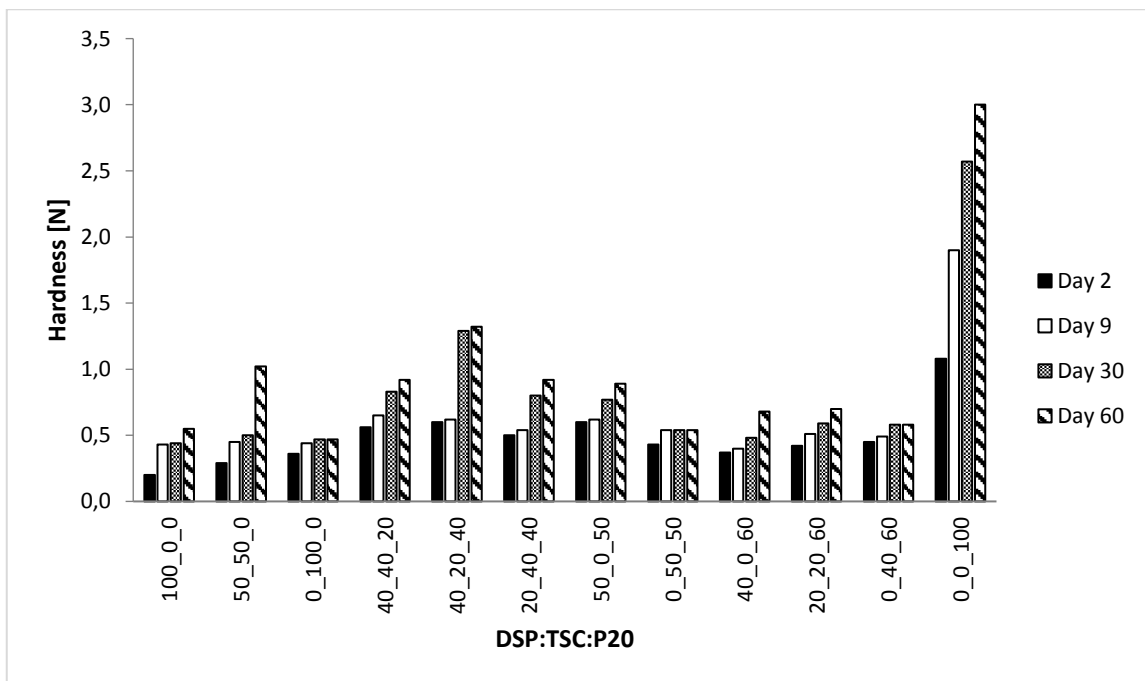

Fig. 13: *pH of model samples of processed cheeses made from a sixteen-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage duration (60 days)*
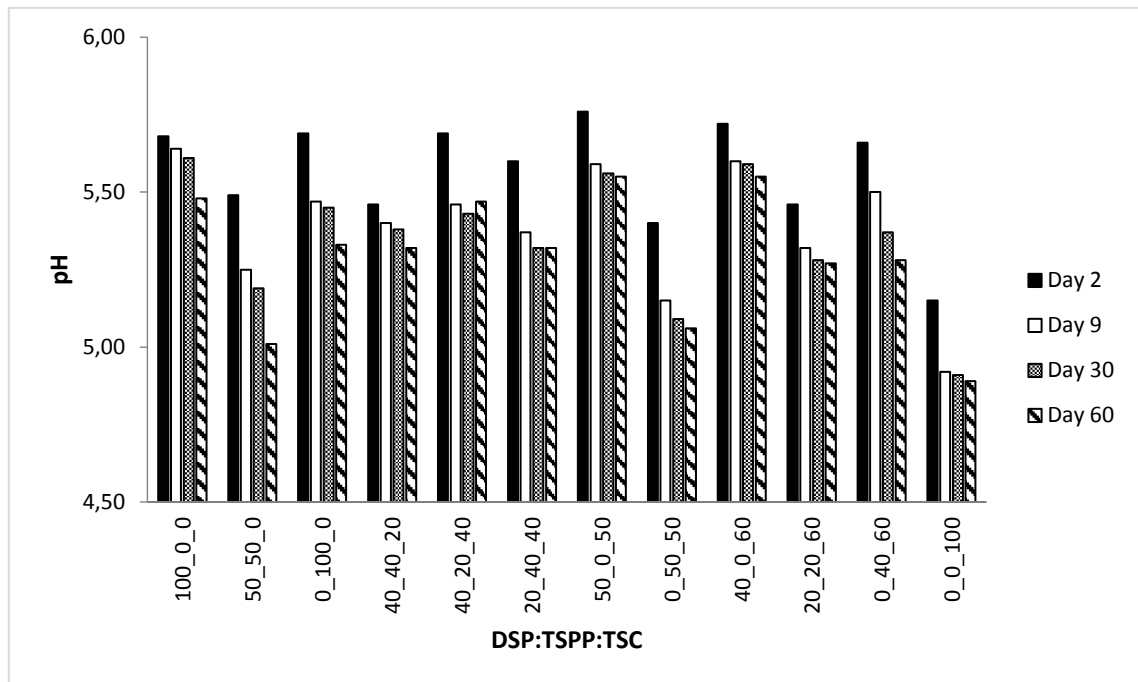
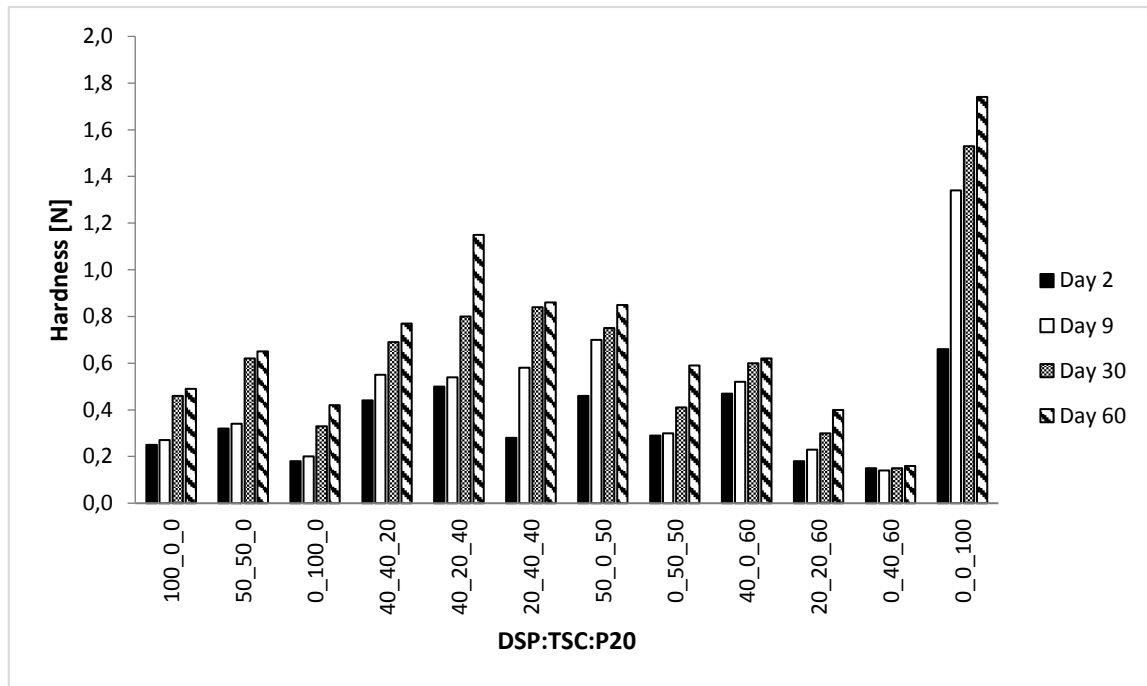***Fig. 14:*** *pH of model samples of processed cheeses made from a twenty four-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage duration (60 days)*

In the following graphs (Figures 15 – 19) the pH values of the model samples of PC are expressed using the TSC:TSPP:P20 ternary mixture.
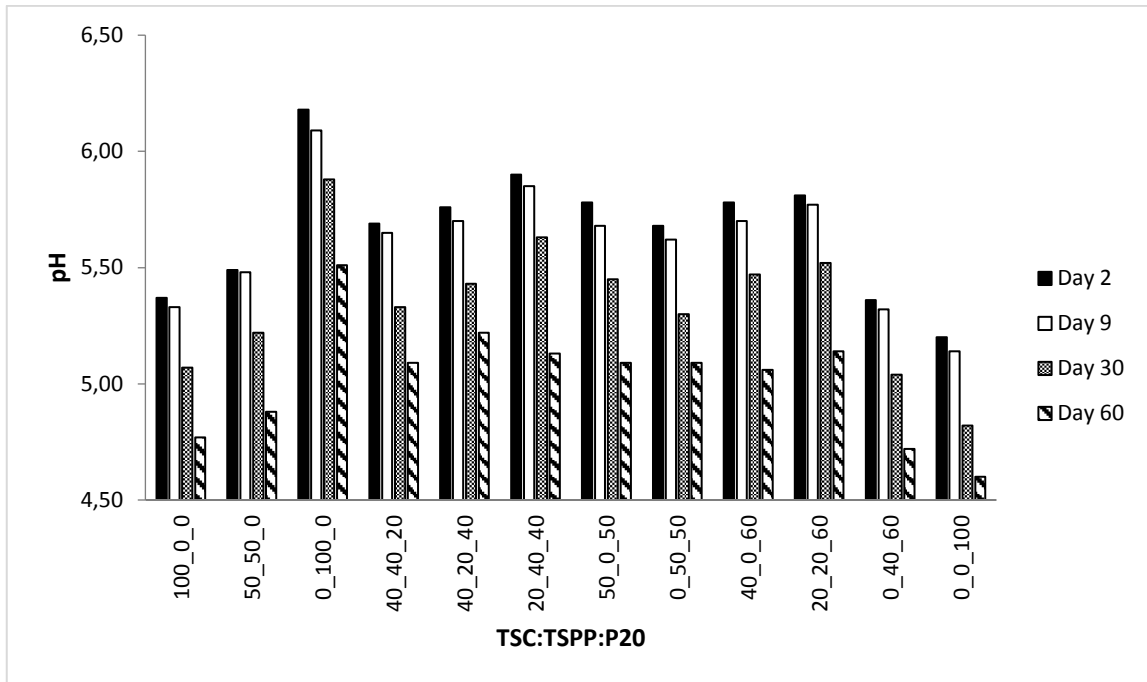
Fig. 15: pH of model samples of processed cheeses made from a two-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage duration (60 days)
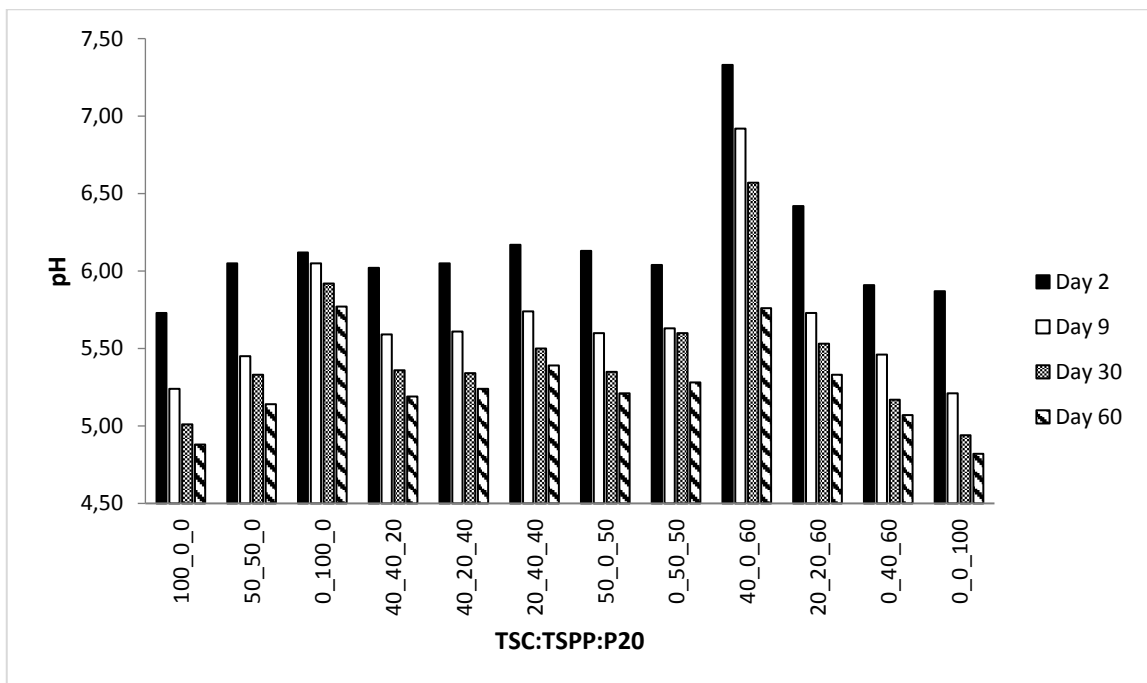
Fig. 16: pH of model samples of processed cheeses made from a four-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage duration (60 days)
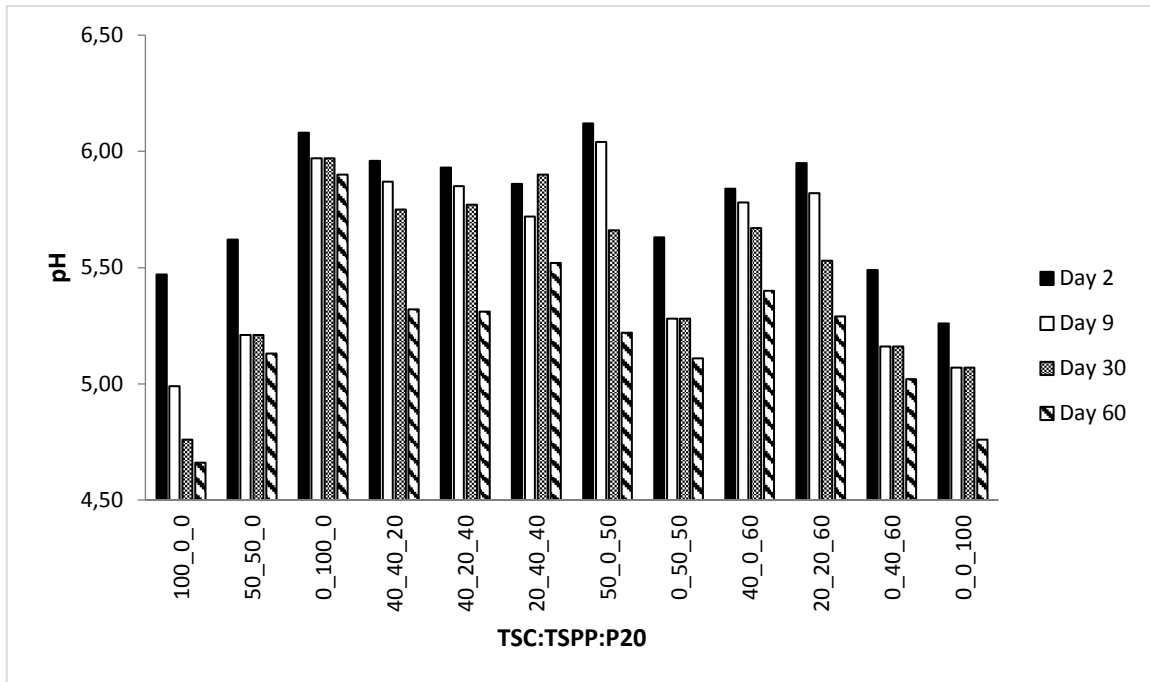
Fig. 17: pH of model samples of processed cheeses made from a eight-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage duration (60 days)

Fig. 18: pH of model samples of processed cheeses made from a sixteen-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage duration (60 days)

Fig. 19: *pH of model samples of processed cheeses made from a twenty four-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage duration (60 days)*

According to the figures above, the pH of the model samples of PC with an increasing amount of TSPP mixed with the TSC and with zero concentration of P20 increased. Increasing the amount of P20 in the model samples showed a decreasing trend of pH. For the 40:0:60 ternary mixture used during the processing, a maximum pH value of 7.33 ± 0.02 was observed for the 4-week natural cheese.

In the following figures (Figure 20 – 24), the pH values of the ternary mixture DSP:TSPP:P20 are shown.

Fig. 20: *pH of model samples of processed cheeses made from a two-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage duration (60 days)*

Fig. 21: *pH of model samples of processed cheeses made from a four-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage duration (60 days)*
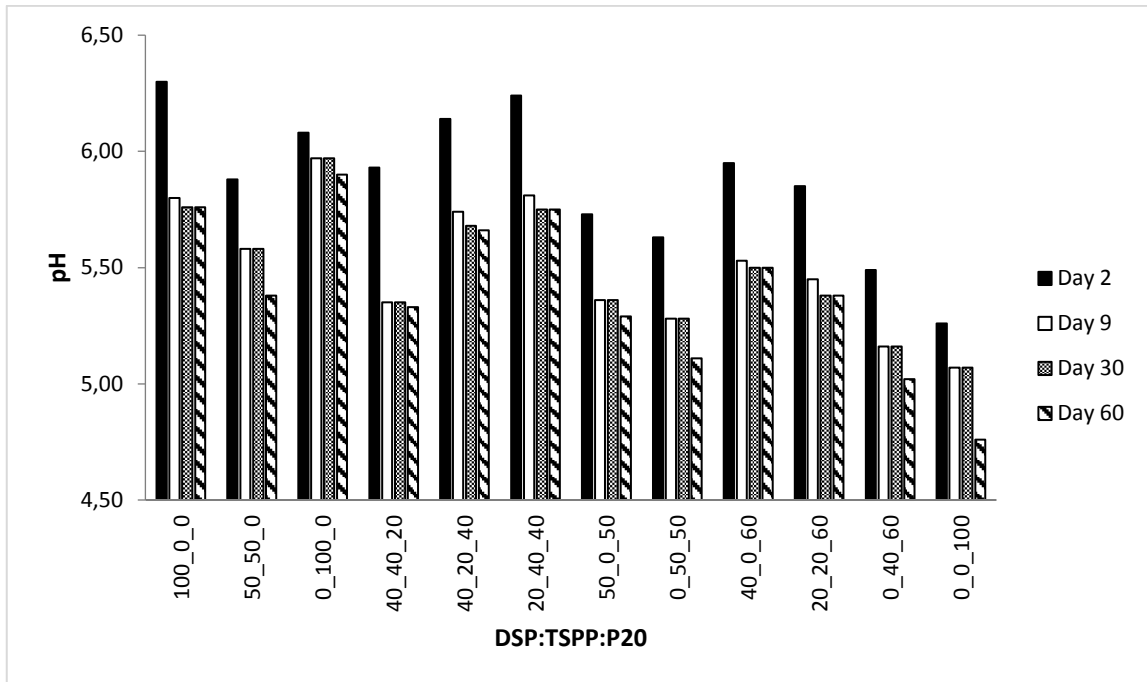
Fig. 22: *pH of model samples of processed cheeses made from a eight-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage duration (60 days)*
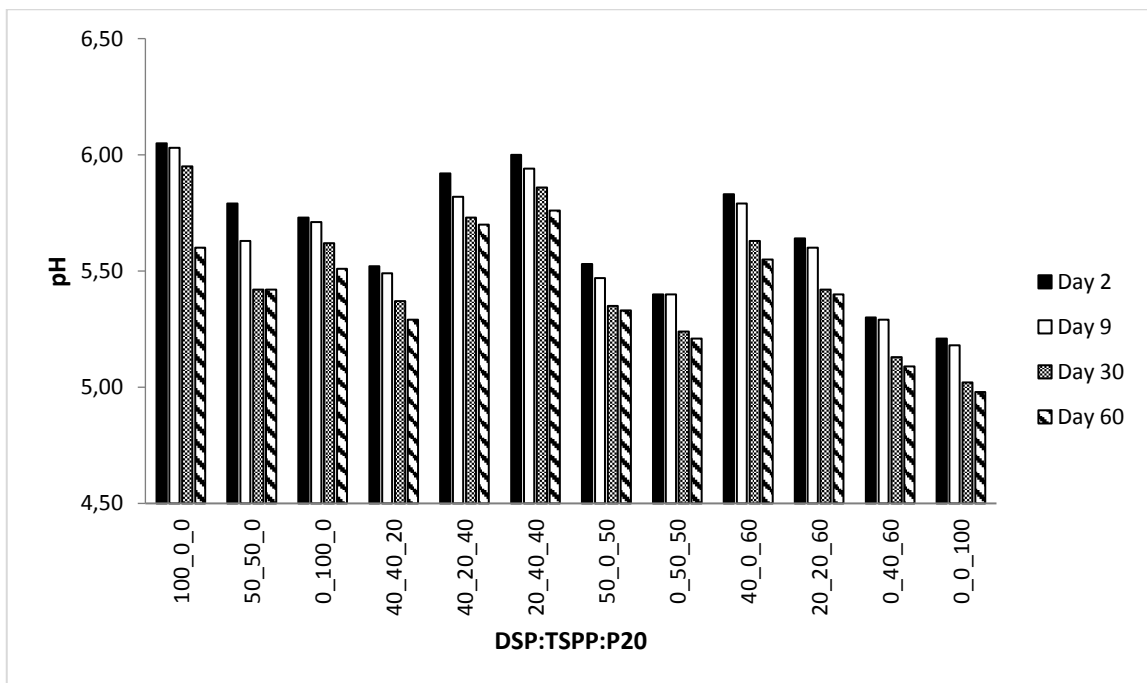
Fig. 23: *pH of model samples of processed cheeses made from a sixteen-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage duration (60 days)*

Fig. 24: *pH of model samples of processed cheeses made from a twenty four-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage duration (60 days)*

Based on the figures above, which characterize the pH of the model samples using the DSP:TSPP:P20 ternary mixture, we can say that during the storage period of the model samples of PC, there was a slight decrease in pH for all samples, irrespective of the maturity level of the applied natural cheese. With 20:40:40 ternary mixture, the pH reached its maximum of 6.58 ± 0.02. Further, we can say with the decreasing content of P20 in the mixture, the pH values increased significantly. Conversely, at 100 % of P20, the minimum pH values were 4.60 ± 0.02.

7.2 Textural profile analysis

7.2.1 Hardness

The hardness results of the model samples of PC are shown in Figures 25 – 44. The following figures present the results of hardness determination using 2, 4, 8, 16 and 24 week raw materials depending on the composition of the ternary mixture and the lenght of the storage period.

Fig. 25: Hardness of model samples of processed cheeses made from a two-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage period (60 days)

Fig. 26: Hardness of model samples of processed cheeses made from a four-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage period (60 days)

Fig. 27: Hardness of model samples of processed cheeses made from a eight-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage period (60 days)
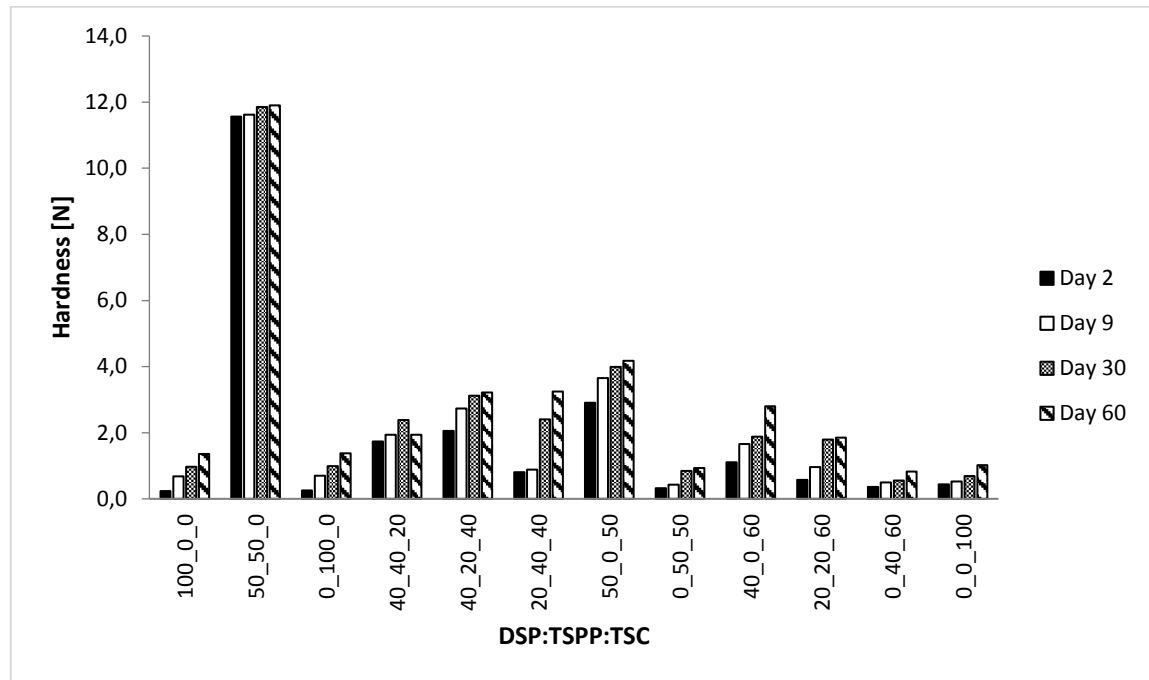

Fig. 28: Hardness of model samples of processed cheeses made from a sixteen-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage period (60 days)
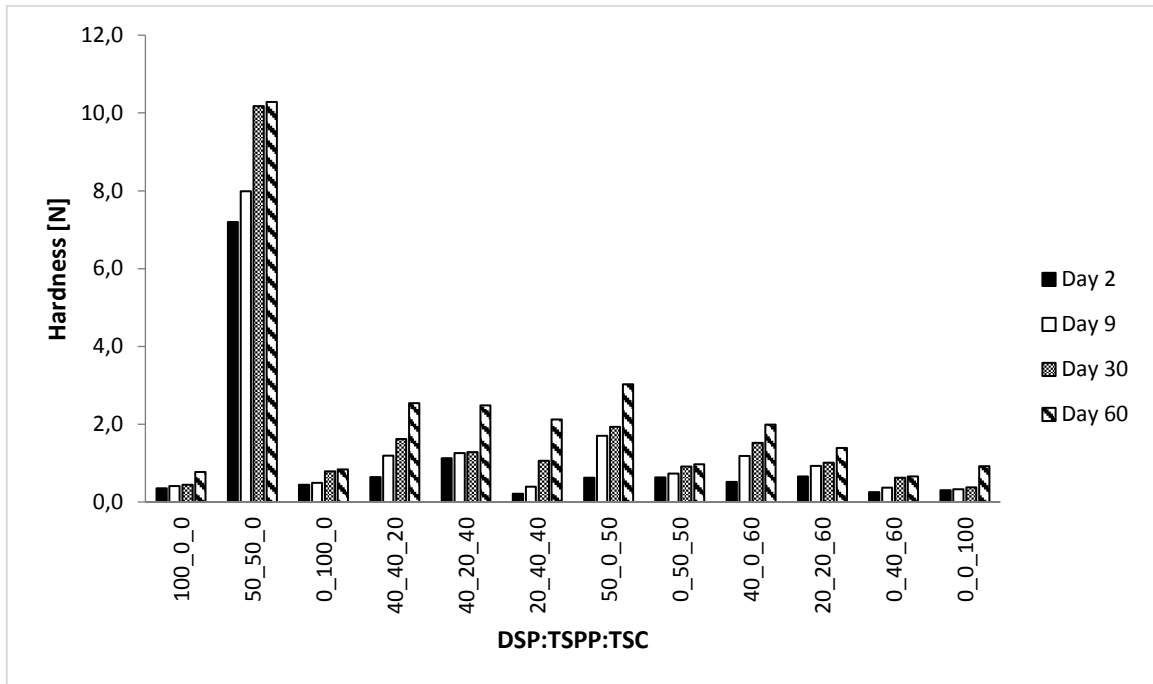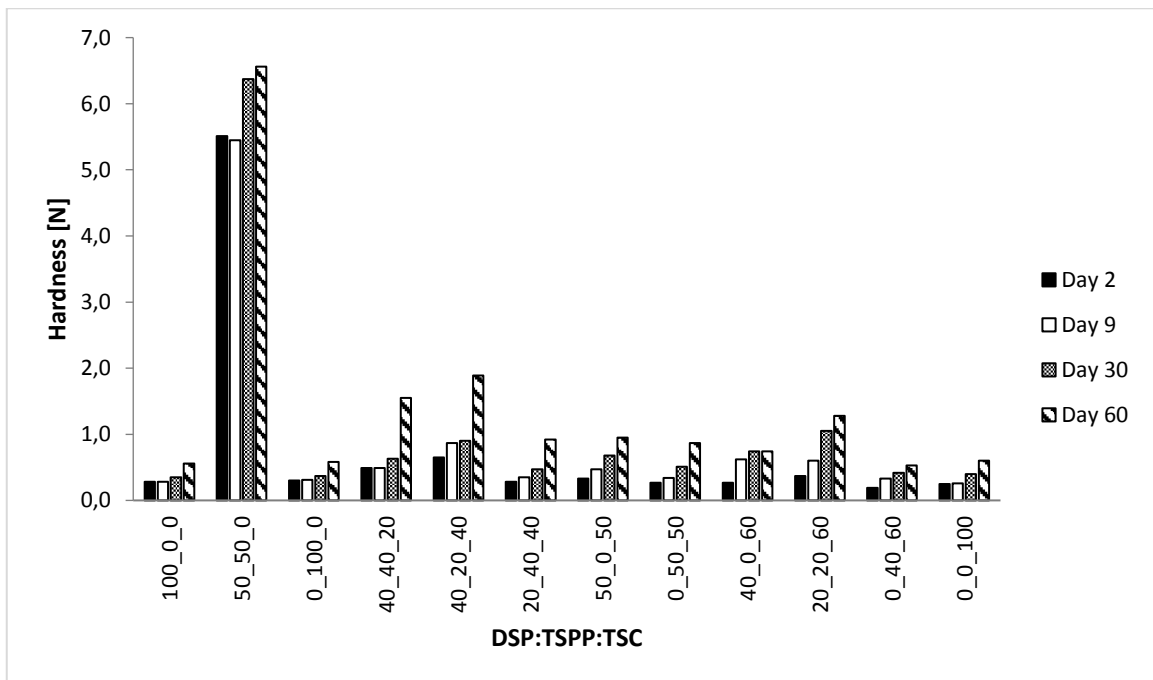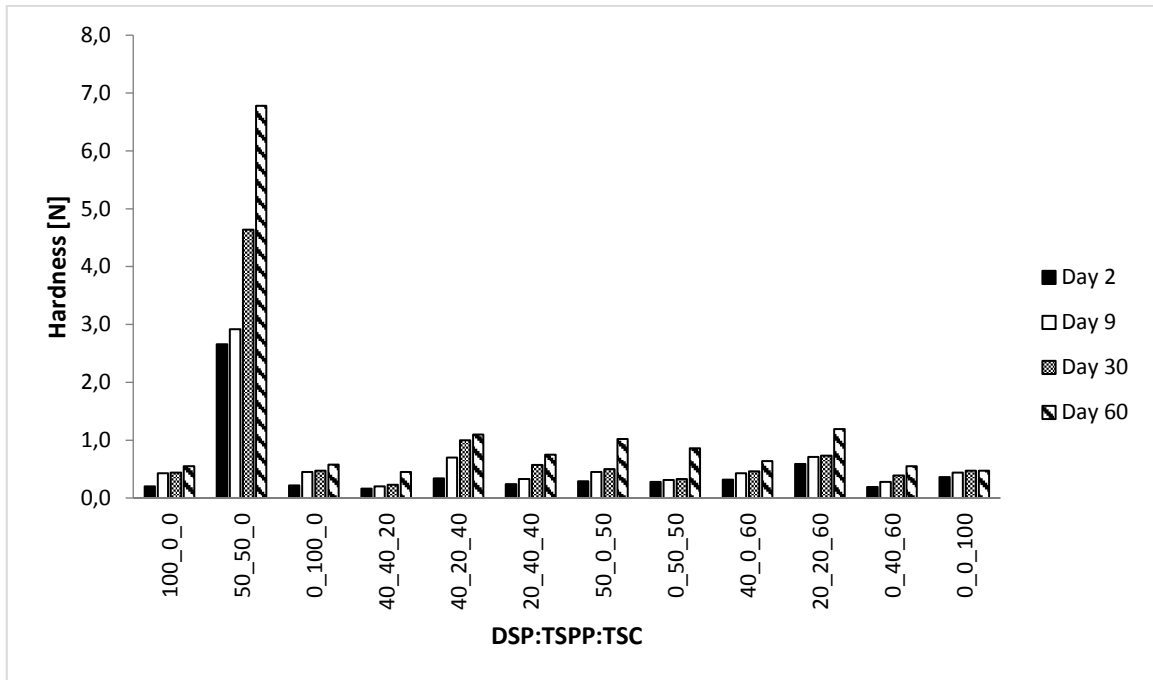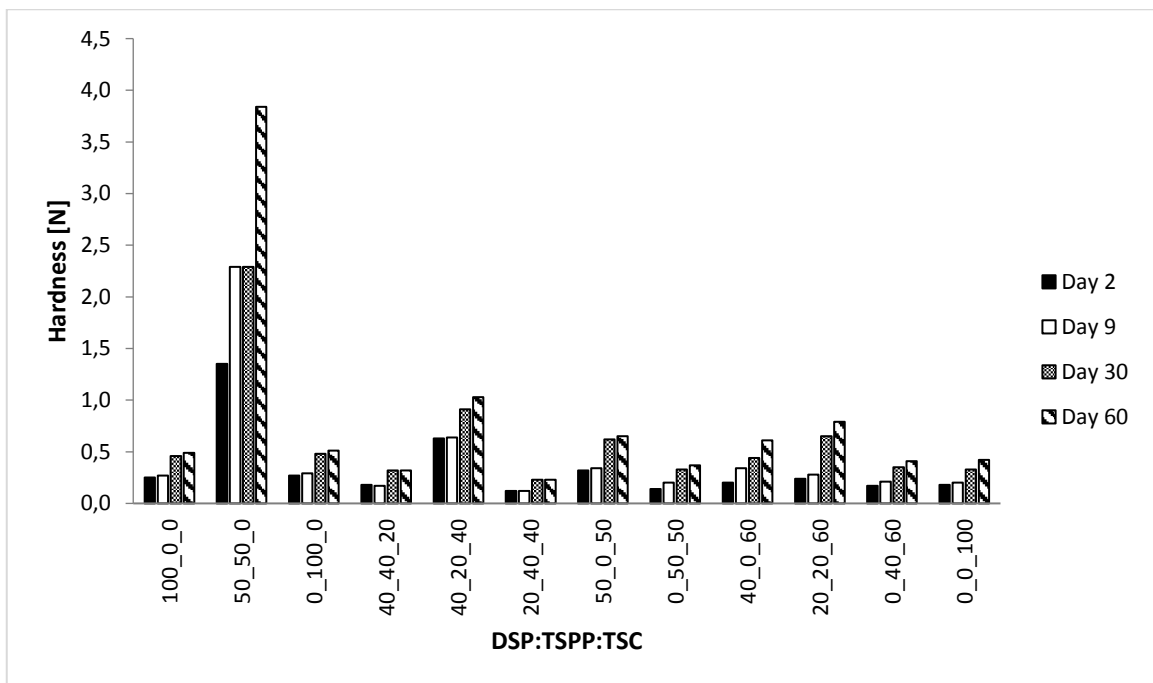
Fig. 29: Hardness of model samples of processed cheeses made from a twenty four-week raw material depending on the composition of the ternary mixture DSP:TSC:P20 and storage period (60 days)

The change in final products consistency is contributed by the hydrolysis of polyphosphate ES used in the production of the model samples of PC. Using a combination of ternary mixture DSP:TSC:P20 (Figure 25 – 29), increasing hardness values were observed during the 60 day storage period. More intense growth of sample hardness was observed between the 2nd and 9th day of storage period, which was due to the more intense hydrolysis of polyphosphates. Decomposition processes begin already during the melting process and continue during storage period. Polyphosphates are progressively hydrolyzed into simple phosphates. As a result of the decrease in the number of $(PO4)^{3-}$ groups in the chain, the affinity for calcium ions decreases, leading to their gradual release. Increased product hardness results from the inclusion of released calcium ions in the protein matrix. The increase in sample hardness values during storage were explained by Mizuno and Lucey [33], Shirashoji et al. [26], Nagyová et al. [5], or Awad et al. [82]. The increase in hardness values during storage period can be explained not only by the hydrolysis of the used ES (with two or more phosphorus atoms in the molecule) but also by possible changes of the bonds of the added ES and their degree of dissociation [5, 26, 33, 84].

Moreover, regardless of the maturity stage of the natural cheese used (as raw material), the maximum values for the final product were observed at the application of the ES P20 as sole ingredient. In the absence of the P20 ES, the final product exhibited the lowest hardness. Minimum hardness values (1.5 ± 0.2 N) were observed at 100 % of the DSP ES. With the increasing content of ES P20 in the mixture, the hardness of the final product also increased. Already from 20 % of P20, the hardness of the final product increased, maintaining a constant trend up to 50:0:50. As mentioned above, the minimum values were observed when DSP ES was applied as sole substance. In the model samples with 100 % of TSC ES, the hardness values over the DSP slightly increased. This phenomenon can be explained by a different degree of dispersion of caseins. In the production of PC, phosphate ES play a very important role in the dispersion of caseins and also in the formation of the final matrix of PC. Mizuno and Lucey stated [85], that the higher the degree of dispersion of caseins, the more intensely these proteins are allowed to show their moisturizing and emulsifying capabilities and at the same time creating a more cross-linked structure [26, 28, 85, 63]. Hence, the higher the degree of crosslinking in the product matrix, the harder the PC can be expected [26, 28, 85, 63]. The authors Mizuno and Lucey [85] further point out, that with the increasing number of phosphors bound in the phosphate molecule (polyphosphate > triphosphate > diphosphate > monophosphate), the degree of casein dispersion increases [85]. Therefore, when delivering P20 individually, thus the latter can provide the hardestt samples.

Comparing the results on the basis of the maturity of the used raw material a decreasing tendency of the hardness values was observed, depending on the increasing maturity of the default raw material. Changes in hardness values depending on the maturity of the default raw material can be explained by the shortening of the chain length of casein fractions due to the wider proteolytic changes occurring in natural cheese during the ripenning process. Proteolysis is one of the most important biochemical phenomenon that takes place in the process of ripening of natural cheeses. During this phenomenon hydrolysis of the protein component occurs on peptides and FFA [13, 86, 87, 88]. Brickley et al. [89] studied the influence of the maturity of natural cheese (Cheddar cheese) on the texture and viscoelastic properties of its resultant PC. The proteolytic rate increased faster within the 28 days of its production. This phenomenon coincided with the highest decrease in the hardness of the PC. The shortening of the length of casein fractions due to ripenning of natural cheese re-

sults in the formation of a less stable structure resulting in a decrease in the hardness of the model samples of PC. This was stated by Brickley et al. [89], Wang et al. [90].

The following graphs (Figure 30 – 34) show the behavior of hardness values when the ternary mixture DSP:TSPP:TSC is used.
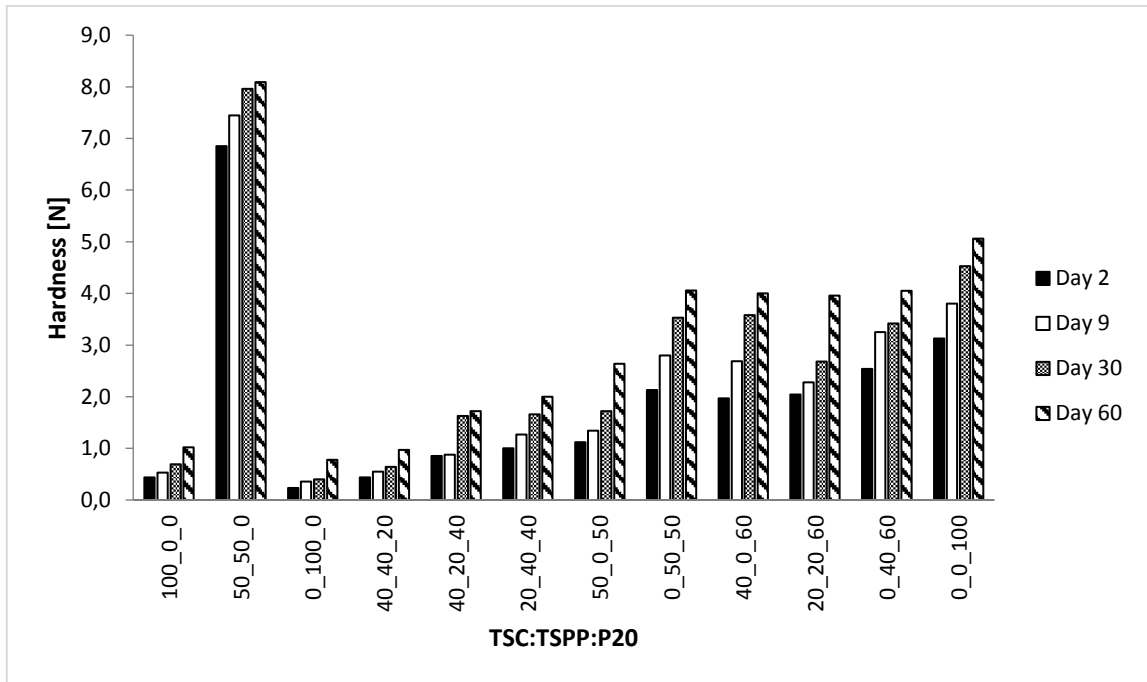

Fig. 30: *Hardness of model samples of processed cheeses made from a two-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage period (60 days)*
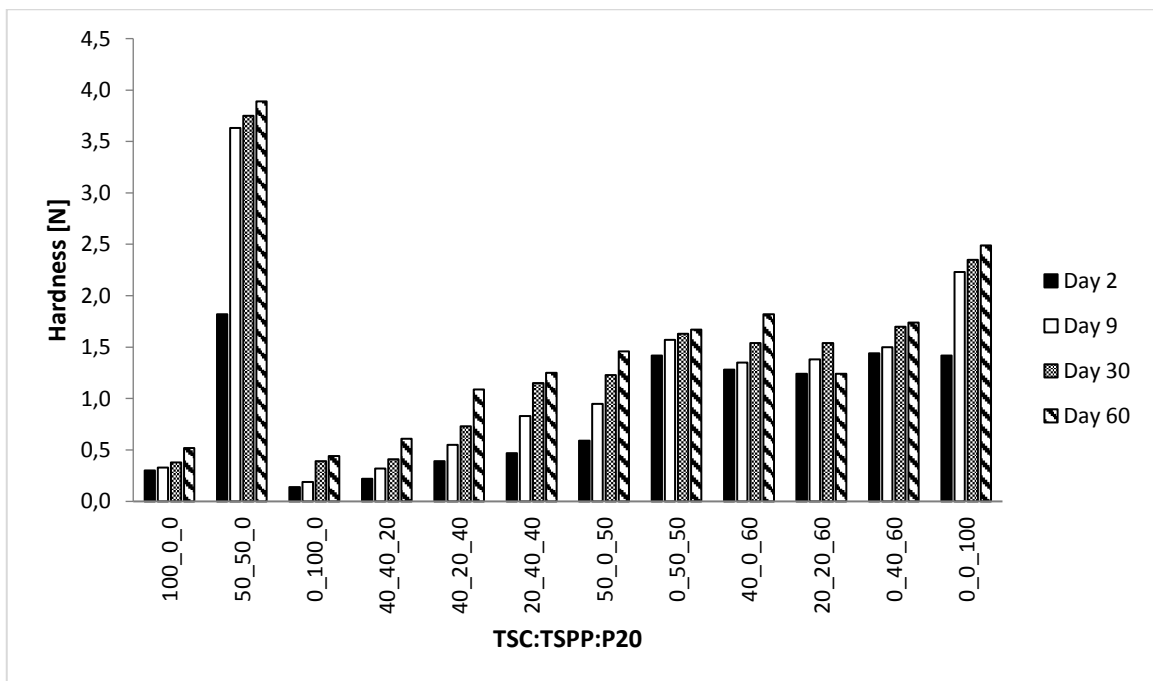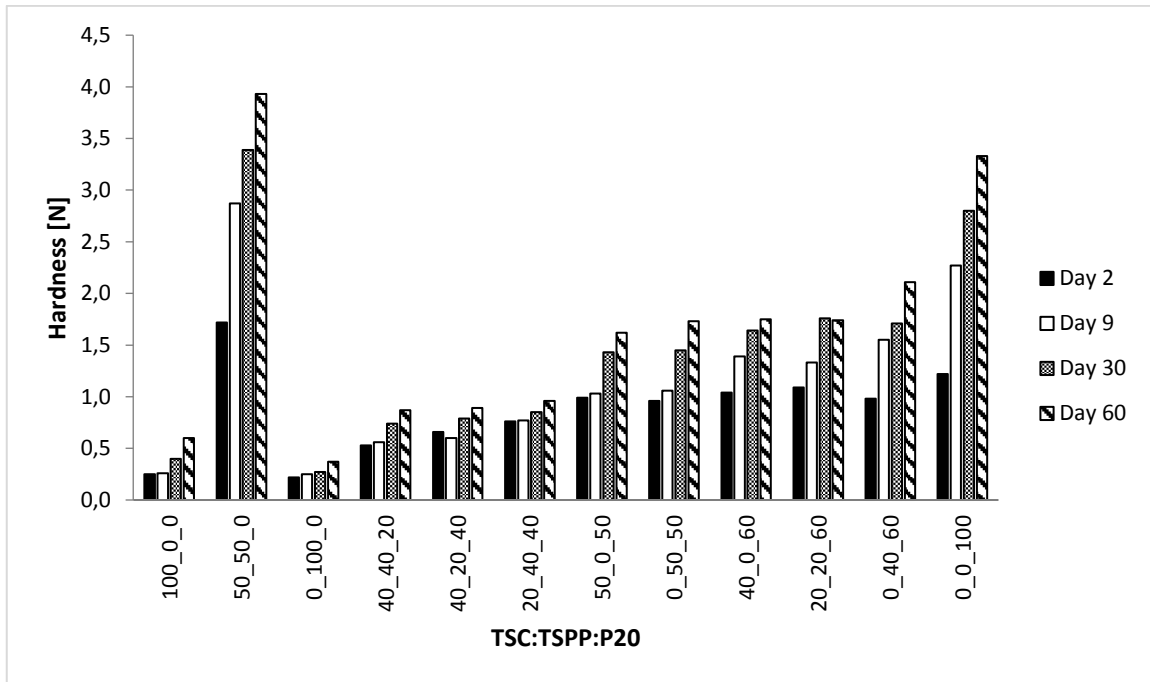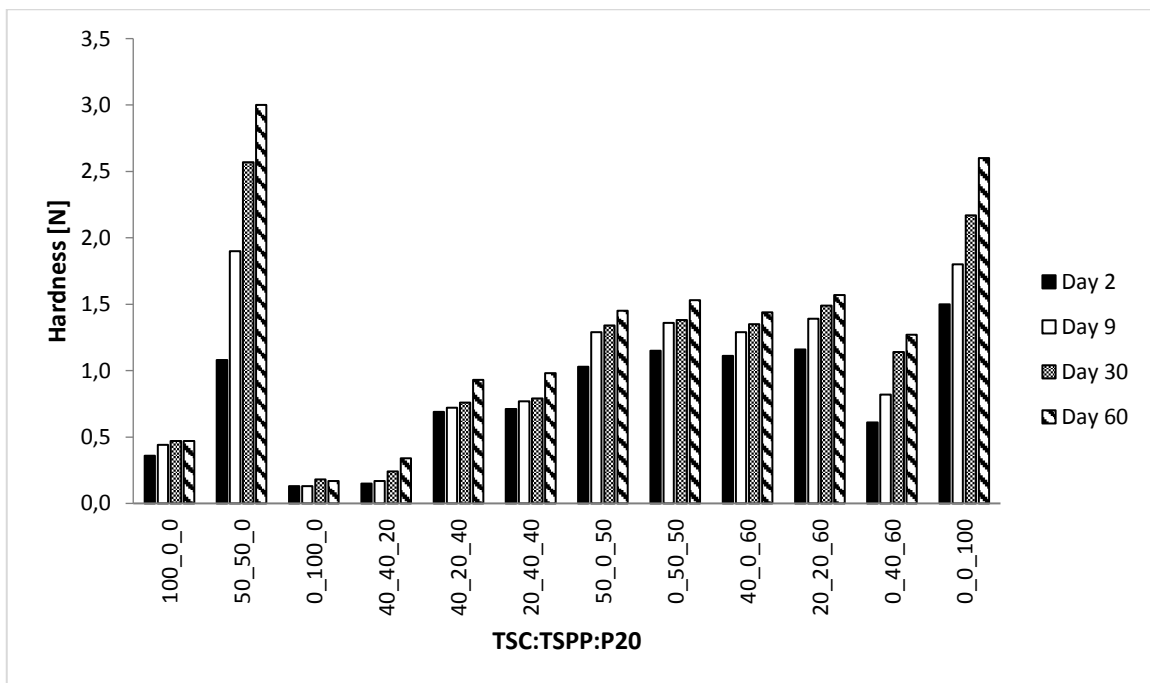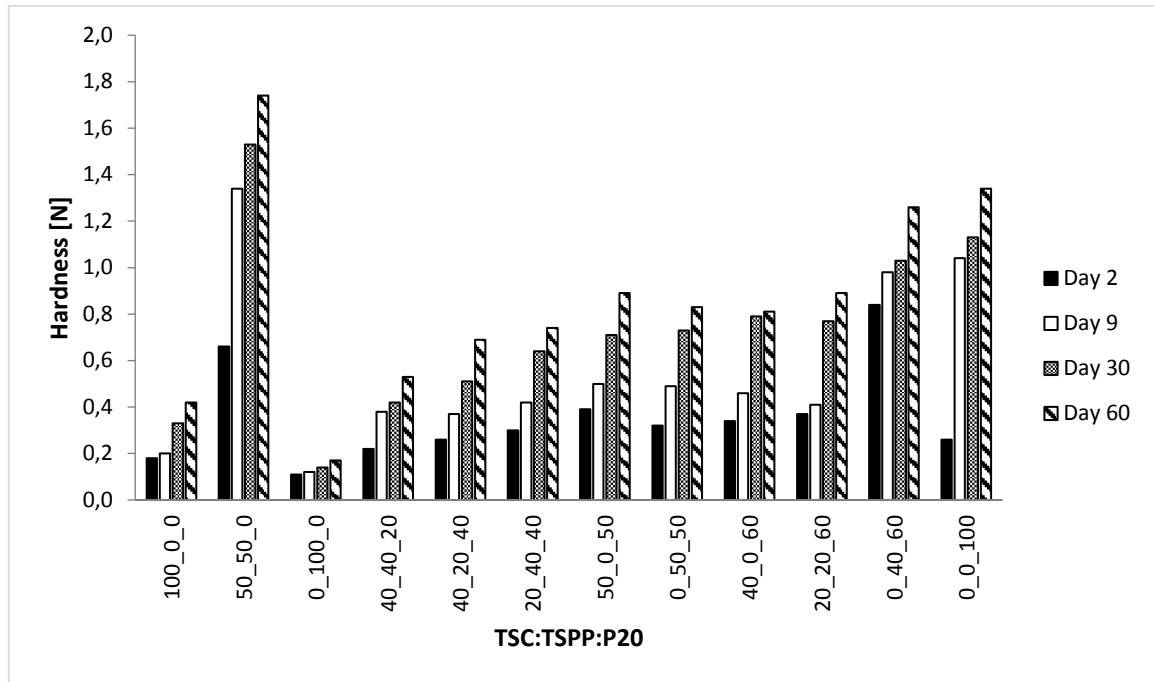
Fig. 31: *Hardness of model samples of processed cheeses made from a four-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage period (60 days)*

Fig. 32: *Hardness of model samples of processed cheeses made from a eight-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage period (60 days)*

Fig. 33: *Hardness of model samples of processed cheeses made from a sixteen-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage period (60 days)*

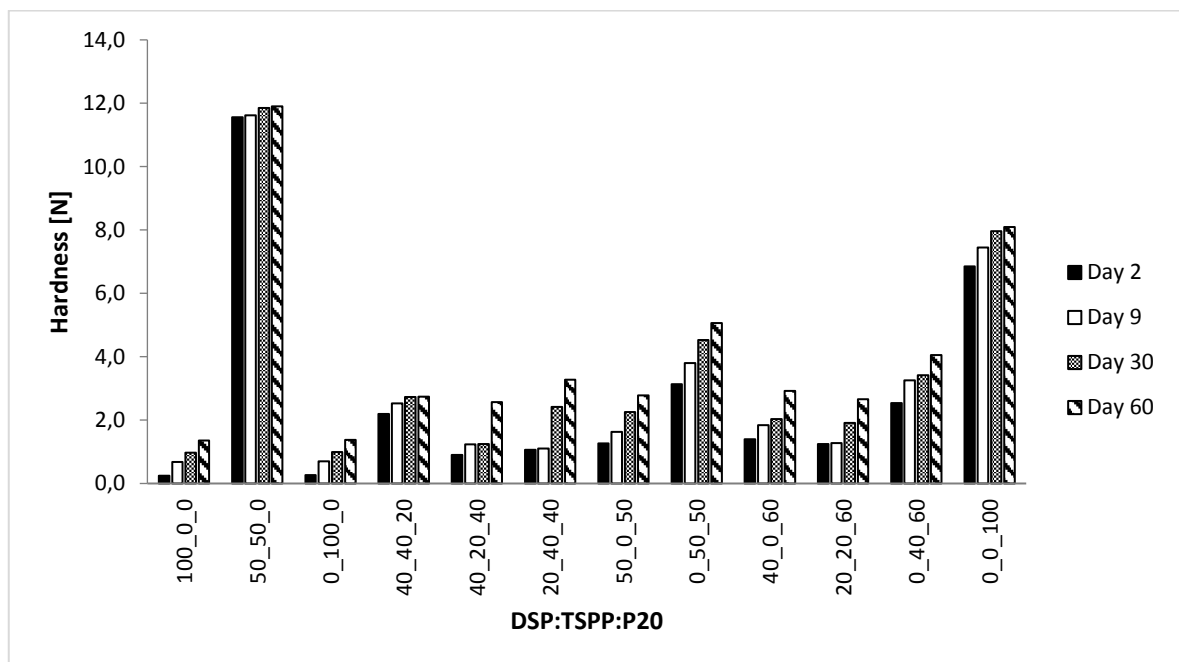*Fig. 34:* *Hardness of model samples of processed cheeses made from a twenty four-week raw material depending on the composition of the ternary mixture DSP:TSPP:TSC and storage period (60 days)*

The following phenomena can be derived from the hardness results when using the DSP:TSPP:TSC ternary mixture (Figure 30 – 34). During the storage of the samples of PC, the hardness values increased again. The lowest hardness of the model samples of PC showed the sample containing 100 % of TSPP salt. Samples with 100 % of DSP and TSC showed similar hardness values. A slight increase in hardness values of samples with a decreasing TSC concentration was observed, particularly in the 40:40:20, 40:20:40, 20:40:40, and 0:50:50 ratios.

Using the ternary mixture of DSP:TSPP:TSC, an interesting trend of hardness development of the model samples of PC was observed. In the absence of TSC in the mixture, maximum hardness values were achieved using a 1:1 mixture of DSP and TSPP. This rapid increase in samples hardness has been observed in the works of Salek et al. [91], Kaliappan and Lucey [92], Shirashoji et al. [26] and Mizuno and Lucey [33]. To clarify the above pheno-menon, the specific properties of the individual phosphates should be taken into account. The reason for the existence of a specific ratio of DSP:TSPP has been found in particular, in the ability of diphosphates to promote the formation of a milk protein gel. The ability of monophosphates to penetrate cross-linking caseins and bind water tightly to the formation of a solid gel [26, 82, 84] is also likely to play a possible role.

Mizuno and Lucey added [33], that there is a certain optimum concentration of diphospha-tes that is capable of efficient gel formation. Moreover, with excessive or insufficient con-centration of diphosphates, weak gels may occur [84]. Kaliappan and Lucey [92] noted, that the mixture of ES composed of monophosphates and diphosphates strongly promotes gel formation in model milk systems which could be explained by the ability of mo-nophosphates to bind onto casein molecules and to promote protein hydration.

In the case of TSC and TSPP content in a 1:1 ratio, a slight increase in hardness was ob-served. According to Mizuno and Lucey [33] and Lu et al. [93], TSC does not bind to caseins and does not interfere with the process of crosslinking the casein matrix. However, by adding TSC, the TSPP concentration may be adjusted to achieve its optimum in order to promote the formation of casein gel [33, 92, 93].

Figures 35 – 39 shows the behavior of hardness values using the ternary mixture of TSC:TSPP:P20.

Fig. 35: *Hardness of model samples of processed cheeses made from a two-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage period (60 days)*
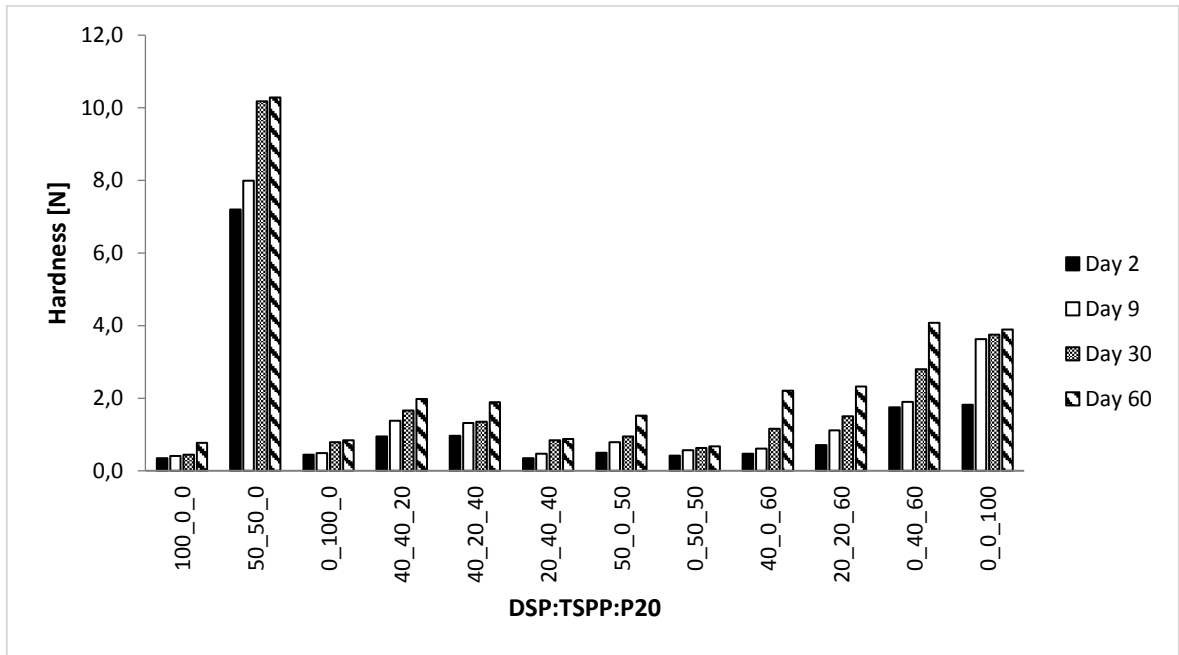

Fig. 36: *Hardness of model samples of processed cheeses made from a four-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage period (60 days)*
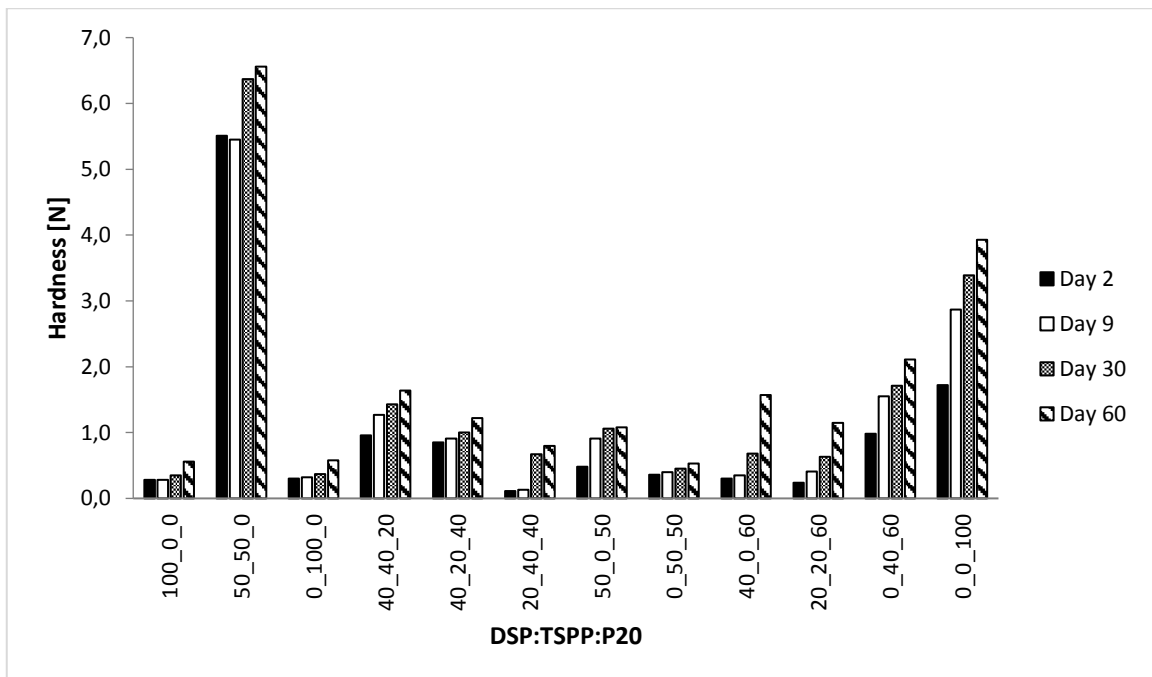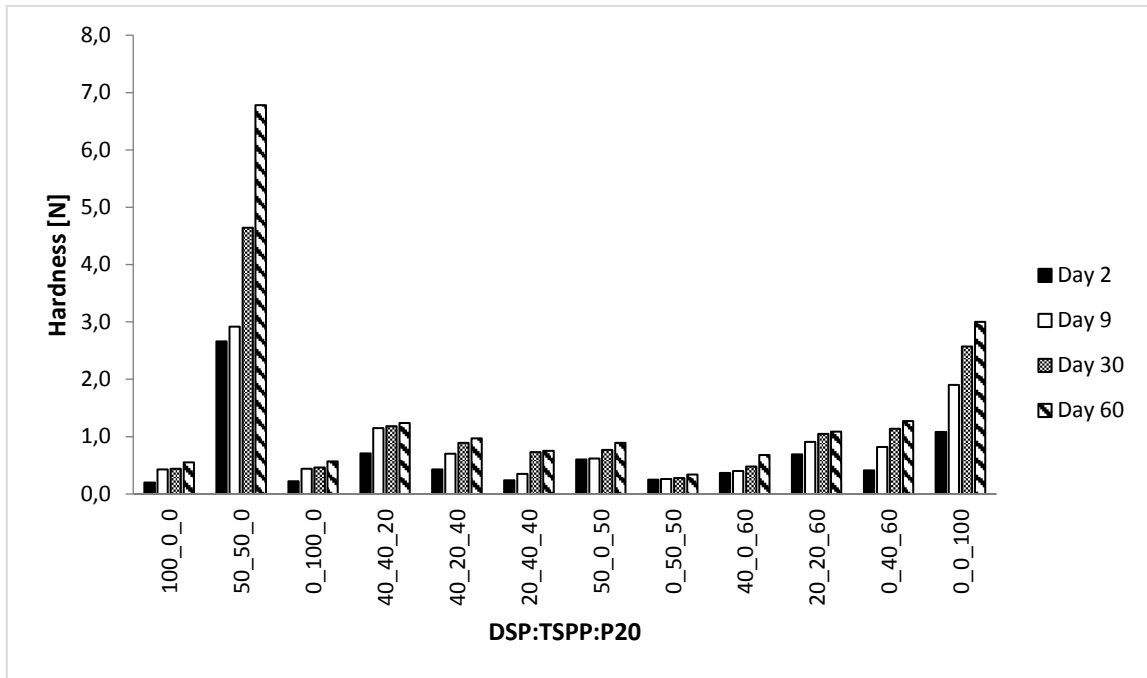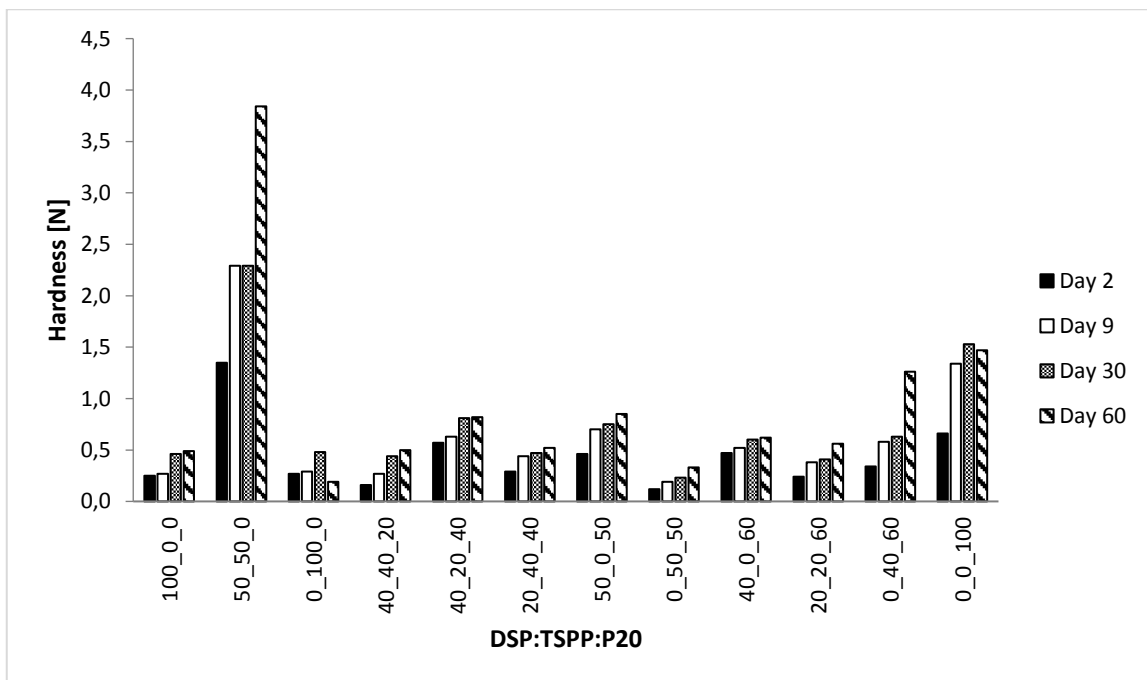
Fig. 37: *Hardness of model samples of processed cheeses made from a eight-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage period (60 days)*

Fig. 38: *Hardness of model samples of processed cheeses made from a sixteen-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage period (60 days)*

Fig. 39: *Hardness of model samples of processed cheeses made from a twenty four-week raw material depending on the composition of the ternary mixture TSC:TSPP:P20 and storage period (60 days)*

When using the TSC:TSPP:P20 ternary mixture (Figures 35 – 39), a similar trend was observed as for the ternary mixture used before (DSP:TSPP:TSC). In the absence of P20 in the mixture, it was observed that from 100 % of TSC in the mixture, the hardness of the PC gradually increased up to a TSC:TSPP ratio of about 1:1 to reach the maximum hardness values. Even this specific ratio had a considerable effect on the textural properties of the model cheese samples. There is no clear explanation for this phenomenon with regard to the interaction between the TSC and TSPP in the examined mixtures. Lu et al. [93], Kaliappan and Lucey [92], Mizuno and Lucey [33] and Salek et al. [91] stated, TSC does not bind to casein proteins and therefore, does not engage in the process of creating a new network. TSC therefore, does not interfere with the effect of diphosphates on cross-linking caseins. A possible explanation, according to Mizuno and Lucey [33], may be the fact that diphosphates promote the formation of the casein protein gel only if they are in optimal concentration relative to the protein content. The addition of TSC at optimal concentration relative to the protein content. By adding TSC, the TSPP concentration can be adjusted to achieve its optimal concentration to support casein gel formation [84].

At 20 % of P20, the hardness of the PC began to grow to 50:0:50, where the growing trend changed into a constant trend. At a 100 % of TSC and a TSPP as sole, minimum hardness values for model samples of PC were found while 100 % of P20 was the maximum. As in the previous cases, the increasing tendency of hardness, depending on the storage time was observed using the TSC:TSPP:P20 ternary mixture and the trend decreasing depending on the maturity of the raw material was once again identified.

In the following figures (Figures 40 – 44) the hardness values of the model samples of PC are expressed using the DSC:TSPP:P20 ternary mixture.

Fig. 40: Hardness of model samples of processed cheeses made from a two-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage period (60 days)

Fig. 41: Hardness of model samples of processed cheeses made from a four-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage period (60 days)

Fig. 42: Hardness of model samples of processed cheeses made from a eight-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage period (60 days)

Fig. 43: *Hardness of model samples of processed cheeses made from a sixteen-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage period (60 days)*

Fig. 44: *Hardness of model samples of processed cheeses made from a twenty four-week raw material depending on the composition of the ternary mixture DSP:TSPP:P20 and storage period (60 days)*

The effects of phosphate ES on the textural properties of PC have already been the subject of many studies, so these figures (Figure 40 – 44) serve only to compare the results. A ternary mixture of DSP:TSPP:P20 ES was used. At a 50:50:0 ratio regardless of the maturity of the raw material, maximum hardness values were observed. With a higher P20 ratio the hardness of all samples decreased by up to 50 %. With a 50:0:50 ratio, the values reported a constant trend. This phenomenon can be explained by the functional properties of the P20 ES. According to Shirashoji et al. [94] and Mizuno and Lucey [84], polyphosphates show the ability to form solid complexes with calcium and sufficient rate of dispersion of caseins. In contrast, they are considered to be inhibitors of the gel. Due to the addition of P20, an increase in the negative charge on the casein chains results in an increase in repellent forces between these polymers and a consequent decrease in hardness values.

Looking at the ES with amount of 100 % in the mixture we could report, that the highest hardness values showed samples with 100 % of P20.

During the 60-day storage period of the model samples of PC, significant hardness changes occurred. As the storage time increased, the hardness of the PC was increased.

7.2.2 Cohesiveness and relative adhesiveness

During the 60-day storage period, samples of PC were observed to increase cohesiveness values and decrease in relative adhesiveness.

With the use of 1:1 DSP:TSPP ES, the cohesiveness and relative adhesiveness values of the model samples of PC decreased. With the increasing addition of P20 in the mixture, this decreasing trend was less intensive.

Moreover, the more mature raw material was used, the lower the cohesiveness values were observed. The same trend was also observed for hardness values. This decrease, with increasing maturity of natural cheese is caused by proteolysis and decay of the protein network of natural cheese [82]. When observing the values of relative adhesiveness with respect to the maturity of raw material, a slight increase was recorded, which is confirmed by the available literature [60, 95].

CONCLUSION

The thesis was focused on the study of the effect of the maturity stage of Akawi White-brined cheese on the consistency of processed cheese with different emulsifying salt composition during a 60-day storage time. The model samples of processed cheeses were made using 4 combinations of ternary mixtures of emulsifying salts that were subjected to basic chemical analysis and textural profile analysis during storage (2nd, 9th, 30th and 60th days).

Based on the measured results it could be reported:

- The dry-matter content of the model samples of processed cheeses ranged from 40.16 – 41.11 % (w/w), corresponded to the target value of the dry-matter content of the samples, which was 40 % (w/w),

- In the individual application of the DSP, TSPP and TSC emulsifying salts, pH values exceeding 6.52 ± 2 were observed,

- At 100 % of P20 application, pH values ranged from 4.60 – 5.87,

- During the 60-day storage period, a slight decrease in the pH of the samples of processed cheese was recorded, regardless to maturity of the raw material,

- At 100 % concentration of the individual emulsifying salts it was found, the highest hardness reached the sample using P20; for the TSPP and TSC the hardness of the model samples was higher than the 100 % DSP representation (P20 > TSPP ~ TSC > DSP)

- With a prolonged maturity period, a decreasing tendency of hardness values was observed irrespective of the combination of the ternary mixture,

- A rapid increase in hardness of the model samples of processed cheeses in the DSP and TSPP emusifying salt in the ratio of 1:1,

- During 60-day storage period, samples of processed cheese were observed to increase cohesiveness values and decrease in relative adhesiveness,

- With a prolonged maturity of the raw material, lower cohesiveness values and increasing relative adhesiveness values were observed.

In general, it could be stated that the hardness of the model samples of processed cheeses was influenced both by the composition of the used ternary mixtures of emulsifying salts, the lenght of the storage period and the maturity degree of the applied raw material.

BIBLIOGRAPHY

[1] TAMIME, A., Processed cheese and analogues. Ames, Iowa: Wiley-Blackwell, 2011, p. 350, Society of Dairy Technology series. ISBN 14-051-8642-9.

[2] BUŇKA, F., BUŇKOVÁ, L. and KRÁČMAR, S. *Základní principy výroby tavených sýrů*, Folia MZLU, Brno, II, 2009. ISBN 978-80-7375-336-8.

[3] Vyhláška Ministerstva zemědělství č. 397/2016 Sb. v platném znění, kterou se stanoví požadavky pro mléko a mléčné výrobky, mražené krémy a jedlé tuky a oleje, v platném znění. *Sbírka zákonů České republiky*.

[4] Draft General Standard for Processed Cheese, Codex Standard 283-1978. Established 1978, Amendment 1 2015. Codex Alimentarius Commission, Joint FAO/WHO food standards programme, Rome, Italy.

[5] NAGYOVÁ, G., BUŇKA, F., SALEK, R.N., ČERNÍKOVÁ, M., MANČÍK, P., GRŮBER, T. and KUCHAŘ, D. Use of sodium polyphosphates with different linear lengths in the production of spreadable processed cheese. *Journal of Dairy Science*. 2014, 97(1), 111-122. DOI: 10.3168/jds.2013-7210. ISSN 00220302.

[6] *Processed Cheese and Analogues*. Oxford, UK: Wiley-Blackwell, 2011. ISBN 9781444341850.

[7] HUI, Y. H. Dairy science and technology handbook. New York, N.Y.: VCH, c1993. ISBN 35-272-8162-2.

[8] GUINEE, T.P., CARIĆ, M. and KALÁB, M., *Pasteurized processed cheese and substitute/imitation cheese products*. s. 349. DOI: 10.1016/S1874-558X(04)800526.

[9] KAPOOR, R., METZGER, E. Process Cheese: Scientific and Technological Aspects - A Review. *Comprehensive Reviews in Food Science and Food Safety*. 2008, **7**(2), 194-214. DOI: 10.1111/j.1541-4337.2008.00040.x. ISSN 1541-4337.

[10] TAMIME, A., Processed cheese and analogues. Ames, Iowa: Wiley-Blackwell, 2011, s. 350, Society of Dairy Technology series. ISBN 14-051-8642-9.

[11] BERGER, W., KLOSTERMEYER, H., MERKENISH, K. and UHLMANN, G., Process cheese manufacture, 1998, B.K. Guilini Chemie GmbH & Co., Ladenberg, Germany.

[12] BUŇKA, F., BUŇKOVÁ, L., ČERNÍKOVÁ, M. and HLADKÁ K. Základní charakteristika tavených sýrů a jejich analogů: *Potravinářská revue.*, 2010, vol. 6, s. 29 - 32. ISSN 1801-9102.

[13] FOX, P. F. and McSWEENEY, P. L. H. *Dairy chemistry and biochemistry*, Chemistry and biochemistry of cheese and fermented milks. Issue 1. Thomson Science, 1998. s. 421 – 428. ISBN 978-0-412-72000-0.

[14] BUŇKA, F., BUŇKOVÁ, L. and KRÁČMAR, S. *Základní principy výroby tavených sýrů*, Folia MZLU, Brno, II, 2009. ISBN 978-80-7375-336-8.

[15] BACHMANN, H. P. Cheese Analogues: A Review. *International Dairy Journal*, 2001, 11, s. 505- 515. ISSN 0958-6946.

[16] CUNHA, C. R., DIAS, A. I. and VIOTTO, W. H. Microstructure, texture, colourand sensory evaluation of a spreadable processed cheese analogue made with vegetable fat. *Food Research International*, 2010, 43, s. 723–729. ISSN 0963-9969.

[17] MARCHESSEAU, S., GASTALDI, E., LAGAUDE, A. and CUQ, J. L. Influence of pH on protein interactions and microstructure of process cheese. *Journal of Dairy Science*, 1997, s. 1483–1489. ISSN 0022- 0302.

[18] GUINEE, T. P. Pasteurized processed cheesse product. *Encyclopedia of dairy science*, 2003, 1, s. 411-418. ISBN 0122272358.

[19] Vyhláška Ministerstva zdravotnictví ČR č. 4/2008 Sb., kterou se stanoví druhy a podmínky použití přídatných látek a extrakčních rozpouštědel při výrobě potravin, v platném znění.

[20] Nařízení Evropského Parlamentu a Rady (ES) č. 1333/2008 o potravinářských přídatných látkách. *Úřední věstník Evropské unie*.

[21] MOLINS, R. A. Phospates in food. CRC Press, Boca Raton, 1991.

[22] ZEHREN, V. L. and NUSBAUM, D. D. Process cheese. 2nd ed. Madison, Wisconsin, USA: Cheese Reporter Publishing, Co, Inc., 2000.

[23] MULSOW, B. B., JAROS, D. and ROHM, H. Processed cheese and cheese analogues. *Structure of Dairy Products*. s. 210–235. Blackwell Publishing Ltd,2007. ISBN 9780470995921.

[24] FOX, P. *Fundamentals of Cheese Science*. 1 vydání. Springer, 2000. 608 s. ISBN 0834212609.

[25] BUŇKA, F. a kol. Úloha tavicích solí při výrobě tavených sýrů. *Potravinářská revue*. 2009. č.1. p. 13-16. 2012.

[26] SHIRASHOJI, N., JAEGGI, J. J. and LUCEY, J. A. Effect of sodium hexameta-phosphate concentration and cooking time on the physicochemical properties of pasteurized process cheese. *Journal of Dairy Science*. 2010, vol. 93, s. 2827 2837. DOI: 10.3168/jds.2009-2960.

[27] AWAD, R. A., ABDEL-HAMID, L. B., EL-SHABRAWY, S. A. and SINGH, R. K. Physical and sensory properties of block processed cheese with formulated emulsifying salt mixtures. *International Journal of Food Properties*. 2004, 7, s. 429-448. ISSN 1094-2912.

[28] EL-BAKRY, M., DUGGAN, E., O´RIORDAN, E. D. and O´SULLIVAN, M. Effects of emulsifying salts reduction on imitation cheese manufacture and functional properties. *Journal of Food Engineering*, 2010, 100, s. 569 – 603. ISSN0260-8774.

[29] DIMITRELI, G. and THOMAREIS, A. S. Instrumental textural and viscoelastic properties of processed cheese as affected by emulsifying salts and in relation to its apparent viskosity. *International Journal of Food Properties*, 2009, s. 261–275. ISSN 1532-2386.

[30] BRICKLEY, C. A. and GOVINDASAMY-LUCEY, S. Influence of emulsifying salts on the textural properties of nongat process cheese made from direct acid cheese bases, *Journal of Dairy Science*, 2008, 91, s. 39–48. ISSN 0022-0302.

[31] LEE, S. K. and KLOSTERMEYER, H. The effect of pH on the rheological properties of reduced-fat model processed cheese spreads, *LWT-Food Sci. Technol.*, 2001, 34 (5), s. 288 – 292. ISSN 0023-6438.

[32] EL-BAKRY, M., DUGGAN, E., O'RIORDAN, E. D. and O'SULLIVAN, M. Effect of chelating salt type on casein hydration and fat emulsification during manufacture and post-manufacture functionality of imitation cheese. *Journal of Food Engineering*. 2011, vol. 102, issue 2, s. 145-153. DOI: 10.1016/j.jfoodeng.2010.08.012.

[33] MIZUNO, R. and LUCEY, J. A. Effects of emulsifying salts on the turbidity and calcium-phosphate-protein interactions in kasein micelles. *Journal of Dairy Science*, 2005, 88, s. 3070-3078. ISSN 0022-0302.

[34] SÁDLÍKOVÁ, I., BUŇKA, F., BUDÍNSKÝ, P., VOLDÁNOVÁ, B., PAVLÍNEK, V. a HOZA, I. The effect of selected phosphate emulsifying salts on viscoelastic properties of processed cheese, *LWT – Food Science and Technology*, 2010, 43, s. 1220 – 1225. ISSN 0023-6438.

[35] ROBINSON, R. K. and TAMIME, A. Y. Feta and related cheeses. New York: Ellis Horwood, 1991. ISBN 0747600775.

[36] MOATSOU, G. and GOVARIS, A. White brined cheeses: A diachronic exploitation of small ruminants milk in Greece. *Small Ruminant Research*. 2011, 101(1-3), 113-121. DOI: 10.1016/j.smallrumres.2011.09.031. ISSN 09214488.

[37] KEMAL ERDEM, Y. Effect of ultrafiltration, fat reduction and salting on textural properties of white brined cheese. *Journal of Food Engineering*. 2005, 71(4), 366-372. DOI: 10.1016/j.jfoodeng.2004.10.040. ISSN 02608774.

[38] KOTCHEN, T. A. Contributions of Sodium and Chloride to NaCl-Induced Hypertension. *Hypertension*. 2005, 45(5), 849-850. DOI: 10.1161/01.HYP.0000164629.94634.27. ISSN 0194-911x.

[39] HEANEY, R. P. Role of Dietary Sodium in Osteoporosis. *Journal of the American College of Nutrition*. 2006, 25(sup3), 271S-276S. DOI: 10.1080/07315724.2006.10719577. ISSN 0731-5724.

[40] MASSEY, L. K. Effect of dietary salt intake on circadian calcium metabolism, bone turnover, and calcium oxalate kidney stone risk in postmenopausal women. *Nutrition Research*. 2005, 25(10), 891-903. DOI: 10.1016/j.nutres.2005.09.017. ISSN 02715317.

[41] PENNER, B., NORM, S., CAMPBELL, R. C., CHOCKALINGAM, A., ZARNKE, K. and VAN VLIET, B. Dietary sodium and cardiovascular outcomes: A rational approach. *Canadian Journal of Cardiology*. 2007, 23(7), 567-572. DOI: 10.1016/S0828-282X(07)70802-4. ISSN 0828282x.

[42] FOX, P. F. Proteolysis during cheese manufacture and ripening. *Journal of Dairy Science*. 1989, 72, 1379–1400.

[43] HAYALOGLU, GUVEN, A. M., FOX, P. F., HANNON, J. A. and MCSWEENEY, P. L. H. Proteolysis in Turkish White-brined cheese made with defined strains of Lactococcus. *International Dairy Journal*. 2004, 14(7), 599-610. DOI: 10.1016/j.idairyj.2003.12.008. ISSN 09586946.

[44] GEORGALA, A., MOSCHOPOULOU, E., AKTYPIS, A., MASSOURAS, T., ZOIDOU, E., KANDARAKIS, I., ANIFANTAKIS, E. Evolution of lipolysis during the ripening of traditional Feta cheese. *Food Chemistry*. 2005, 93, 73–80.

[45] GEORGALA, A.K., KAMINARIDES, S.E., ANIFANTAKIS, E.M. Free fatty acid content of some traditional cheese varieties. *Dairy Technology*. 2006, 61, 26–31.

[46] ALICHANIDIS, E., ANIFANTAKIS, E.M., POLYCHRONIADOU, A., NANOU, M. Suitability of some microbial coagulants for Feta cheese manufacture. *Journal of Dairy Research*. 1984, 51, 141–147.

[47] VAFOPOULOU, A., ALICHANIDIS, E., ZERFIRIDIS, G., 1989. Accelerated ripening of Feta cheese, with heat-shocked cultures or microbial proteinases. *Journal of Dairy Research*. 1989, 56, 285–296.

[48] MANOLAKI, P., KATSIARI, M.C., ALICHANIDIS, E. Effect of a common adjunct culture on organic acid contents of low-fat Feta-type cheese. *International Dairy Journal*. 2006, 98, 658–663.

[49] VISSER, F. M. W. Contribution of enzymes from rennet, starter bacteria and milk to proteolysis and flavour development in Gouda cheese. 1. Description of cheese and aseptic cheesemaking techniques. *Netherlands Milk and Dairy Journal*, 1977, 31, 120–133.

[50] FOX, P. F., and MCSWEENEY, P. L. H. Proteolysis in cheese during ripening. *Food Reviews International*, 1996, 12, 457–509.

[51] RANK, T. C., GRAPPIN, R., and OLSON, N. F. Secondary proteolysis of cheese during ripening: A review. *Journal of Dairy Science*, 1985, 68, 801–805.

[52] FOX, P. F., LAW, J., MCSWEENEY, P. L. H., & WALLACE, J. Biochemistry of cheese ripening. In P. F. Fox (Ed.), Cheese: Chemistry, physics and microbiology, Vol. 1 (pp. 389–438). 1993, London: Chapman & Hall.

[52] BOCKELMANN, W. The proteolytic system of starter and nonstarter bacteria: Components and their importance for cheese ripening. *International Dairy Journal*, 1995, 5, 977–994.

[53] LAW, B. A. Flavor development in cheese. In F. L. Davies, & B. A. Law (Eds.), Advances in the microbiology and biochemistry of cheese and fermented milk (pp. 187–208). 1984, London: Elsevier Applied Science.

[54] AYYASH, M.M., SHERKAT, F. and SHAH, N.P. The effect of NaCl substitution with KCl on Akawi cheese: Chemical composition, proteolysis, angiotensin-converting enzyme-inhibitory activity, probiotic survival, texture profile, and sensory properties. *Journal of Dairy Science*. 2012, 95(9), 4747-4759. DOI: 10.3168/jds.2011-4940. ISSN 00220302.

[55] McSWEENEY, P. L. H. Biochemistry of cheese ripening. *International Journal of Dairy Technology*. 2004, 57(2-3), s. 127–144. DOI: 10.1111/j.1471 0307.2004.00147.x.

[56] TURNER K. W. and THOMAS T. D., Lactose fermentation in Cheddar cheese and the effect of salt. *New Zealand Journal of Dairy Science and Technology*, 1980. p. 265–276. ISSN 0300 – 1342.

[57] PARENTE E. and COGAN T. M., Starter cultures: general aspects. In Cheese: chemistry, physics and microbiology, Vol 1: General Aspects, 3rd edn, Fox P F, McSweeney P. L. H., Cogan T M and Guinee T. P, eds. London: Elsevier. 2004. s. 123–148. DOI: 10.1016/S1874-558X(04)80065-4.

[58] MCSWEENEY P. L. H. and FOX P. F., Metabolism of residual lactose and of-lactate and citrate. In Cheese: chemistry, physics and microbiology, Vol 1: General Aspects, 3rd edn,. Fox P F, McSweeney P L H, Cogan T M and Guinee T P, eds. London: Elsevier. 2004. s. 361–372. DOI: 10.1016/S1874-558X(04)80074-5.

[59] KAPOOR, R., METZGER, L. E., BISWAS, A. C. and MUTHUKUMMARAPPAN, K. Effect of natural cheese characteristics on process cheese properties. *Journal of Dairy Science*. 2007, vol. 90, issue 4, s. 1625 - 1634. DOI: 10.3168/jds.2006-746.

[60] CARIĆ, M. and KALÁB, M. *Cheese: chemistry, physics and microbiology*, Vol. 2, Major Cheese Groups, 2. vydání. P.F.Fox, ed., Chapman & Hall, London, 1997, s. 467-505. Processed cheese products. ISBN 0-412-535106.

[61] BUŇKOVÁ, L., PLEVA, P., BUŇKA, F., VALÁŠEK, P. and KRÁČMAR, S. Antibacterial effects of commercially available phosphates on selected microorganisms. *Acta univ. agric. et silvic. Mendel. Bru.*, 2008, LVI, 5, s. 1–6. ISSN 211-8516.

[62] GUINEE, T. P., CARIĆ, M. and KALÁB, M. *Cheese: chemistry, physics and microbiology*. Vol. 2, Major cheese Gross. 3. vydání. In. Fox, P.H. (Ed.), London: Elsevier, 2004, s. 349-394. Pasteurized processed cheese and substitute/Imitation cheese products. ISBN 0-1226-3653-8.

[63] SHIRASHOJI, N., JAEGGI, J. J. and LUCEY J. A. Effect of trisodium citrate-concentration and cooking time on the physicochemical properties of pasteurized-process cheese. *Journal of Dairy Science*. 2006, vol. 89, s. 15 – 28. DOI: 10.3168/jds.s0022-0302(06)72065-3.

[64] ABDEL-HAMID, L. B., El-SHABRAWY, S. A. and AWAD, R. A. Chemical properties of processed ras cheese spreads as affected by emulsifying salt mixtures. *Journal of Food Processing and Preservation*, 2000, 24, s. 191–208. ISSN 1745-4549.

[65] PHILLIPS, G. O. and WILLIAMS, P. A. *Handbook of hydrocolloids*. Boca Raton, FL: CRC Press, 2000. ISBN 08-493-0850-x.

[66] VELÍŠEK, J. and HAJŠLOVÁ, J. *Chemie potravin*. Rozš. a přeprac. 3. vyd. Tábor: OSSIS, 2009. ISBN 978-80-86659-17-6.

[67] TAN, Y.L., YE, A., SINGH, H. and HEMAR, Y. Effects of Biopolymer Addition on the Dynamic Rheology and Microstructure of Renneted Skimmed Milk Systems. *Journal of Texture Studies*. 2007, 38(3), 404-422. DOI: 10.1111/j.1745-4603.2007.00104.x. ISSN 0022-4901.

[68] LU, Y., NOBUAKI, N. and LUCEY, J. Rheological, textural and melting properties of commercial samples of some of the different types of pasteurized processed cheese. *International Journal of Dairy Technology*. 2007, 60(2), 74-80. DOI: 10.1111/j.1471-0307.2007.00314.x. ISSN 1364-727x.

[69] MACKŮ, I., BUŇKA, F., VOLDÁNOVÁ B and PAVLÍNEK, V. Effect of addition of selected solid cosolutes on viscoelastic properties of model processed cheese containing pectin. *Food Hydrocolloids*. 2009, 23(8), 2078-2084. DOI: 10.1016/j.foodhyd.2009.03.020. ISSN 0268005x.

[70] ČERNÍKOVÁ, M., BUŇKA, F., PAVLÍNEK, V., BŘEZINA, P., HRABĚ, J. and VALÁŠEK, P. Effect of carrageenan type on viscoelastic properties of processed cheese. *Food Hydrocolloids*. 2008, 22(6), 1054-1061. DOI: 10.1016/j.foodhyd.2007.05.020. ISSN 0268005x.

[71] ČERNÍKOVÁ, M., BUŇKA, F., POSPIECH, M., TREMLOVÁ, B., HLADKÁ, K., PAVLÍNEK V. and BŘEZINA, P. Replacement of traditional emulsifying salts by selected hydrocolloids in processed cheese production. *International Dairy Journal*. 2010, 20(5), 336-343. DOI: 10.1016/j.idairyj.2009.12.012. ISSN 09586946.

[72] SYRBE, A., BAUER, W.J. and KLOSTERMEYER, H. Polymer Science Concepts in Dairy Systems — an Overview of Milk Protein and Food Hydrocolloid Interaction. *International Dairy Journal*. 1998, 8(3), 179-193. DOI: 10.1016/S0958-6946(98)00041-7. ISSN 09586946.

[73] LEE, S. K., ANEMA, S. and KLOSTERMEYER, H. The influence of moisture content on the rheological properties of processed cheese spreads. *International Journal of Food Science and Technology*. 2004, 39(7), 763-771. DOI: 10.1111/j.1365-2621.2004.00842.x. ISSN 0950-5423.

[74] FORDE, A. and FITZGERALD, G.F. Biotechnological approaches to the understanding and improvement of mature cheese flavour. *Current Opinion in Biotechnology*. 2000, 11(5), 484-489. DOI: 10.1016/S0958-1669(00)00130-0. ISSN 09581669.

[75] ČERNÍKOVÁ, M., SALEK, R., N., KOZÁČKOVÁ, D., BĚHALOVÁ, H., LUŇÁKOVÁ, L. and BUŇKA, F. The effect of selected processing parameters on viscoelastic properties of model processed cheese spreads. *International Dairy Journal*. 2017, 66, 84-90. DOI: 10.1016/j.idairyj.2016.11.007. ISSN 09586946.

[76] NORONHA, N., O'RIORDAN, D. and O'SULLIVAN, M. Influence of processing parameters on the texture and microstructure of imitation cheese. *European Food Research and Technology*. 2008, 226(3), 385-393. DOI: 10.1007/s00217-006-0549-9. ISSN 1438-2377.

[77] ANONYM. ČSN EN ISO 5534. *Sýry a tavené sýry – Stanovení obsahu celkové sušiny (Referenční metoda)*. Český normalizační institut, Praha, 2005, 12 s. Třídící znak 571003

[78] ANONYM. Křivka texturní profilové analýzy. Software Texture Exponent Lite (Stable Micro Systéme, Ldt.)

[79] SZCZESNIAK, A. S. Texture is a sensory property. *Food Quality and Preference*. 2002, vol. 13, issue 4, p. 215-225. DOI: 10.1016/S0950-3293(01)00039-8.

[80] NORONHA, N., O'RIORDAN, E. D. and O'SULLIVAN, M. Replacement of fat with functional fibre in imitation cheese. *International Dairy Journal*, 17, 2007. p. 1073-1082. DOI: 10.1016/j.idairyj.2007.01.011.

[81] WEISEROVÁ, E., DOUDOVÁ, L., GALIOVÁ, L., ŽÁK, L., MICHÁLEK, J., JANIŠ, R. a BUŇKA, F. The effect of combinations of sodium phosphates in b nary mixtures on selected texture parameters of processed cheese spreads. *International Dairy Journal*. 2011, vol. 21, issue 12, p. 979-986. DOI: 10.1016/j.idairyj.2011.06.006.

[82] LU, Y., SHIRASHOJI, N. and LUCEY, J. A. Effects of pH on the textural properties and meltability of pasteurized process cheese made with different types of emulsifying salts. *Journal of Food Science*, 2008. p. 363 - 369. DOI: 10.1111/j.1750-3841.2008.00914.x.

[83] AWAD, R. A., ABDEL-HAMID, L. B., EL-SHABRAWY, S. A. and SINGH, R. K. Texture and microstructure of block type processed cheese with formulated emulsifying salt mixtures. *LWT-Food Science and Technology*, 2002, 35, p. 54-61. ISSN 0023-6438.

[84] MIZUNO, R. and LUCEY, J. A. Properties of milk protein ges formed by phosphates. *Journal of Dairy Science*, 2007, 90, p. 452–4531. ISSN 0022-0302.

[85] MIZUNO, R. and LUCEY, J. A. Effects of two types of emulsifying salts on the functionality of nonfat pasta filata cheese. *Journal of Dairy Science*, 2005, 88, p. 3411–3425. ISSN 0022-0302.

[86] SCOTT, R., ROBINSON, R. and WILBEY, R. *Cheesemaking practice*. 3rd ed. Gaithersburg, Md.: Aspen Publication, 1998, xxiii, s. 449. ISBN 07-514-0417-9.

[87] CHANDAN, R. C., KILARA, A. and SHAH, N. *Dairy processing and quality assurance*. Ames, Iowa: Wiley-Blackwell, 2008, xii, s. 586. ISBN 08-138-2756-6.

[88] FOX, P. F. *Cheese: chemistry, physics and microbiology*. 3rd ed. Amsterdam: Elsevier, 2004, xi, s. 434. ISBN 0-1226-3653-82.

[89] BRICKLEY, C. A., AUTY, M. A. E., PIRAINO, P. and McSWEENEY, P. L. H. The effect of cheddar cheese ripening on the functional and textural properties of the processed cheese manufactured therefrom. *Journal of Food Science*, 2007, 72, p. 483–490. ISSN 0022-1147.

[90] WANG, F., ZHANG, X., LUO, J., GUO, H., ZENG, S. S. and REN, F. Effect of proteolysos and calcium equilibrium on functional properties of natural cheddar cheese during ripening and the resultant processed cheese. *Journal of Food Science*, 2011, 76, p. 248–253. ISSN 0022-1147.

[91] SALEK, R. N., ČERNÍKOVÁ, M., NAGYOVÁ, G., KUCHAŘ, D., BAČOVÁ, H., MINARČÍKOVÁ, L. and 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, vol. 44, p. 37-43. DOI: 10.1016/j.idairyj.2014.12.009.

[92] KALIAPPAN, S. and LUCEY, J. A. Influence of mixtures of calcium-chelating salts on the physicochemical properties of casein micelles. *Journal of Dairy Science*, 94, 2011. p. 4255-4263. DOI: 10.3168/jds.2010-3343.

[93] LU, Y., SHIRASHOJI, N. and LUCEY, J. A. Effects of pH on the textural properties and meltability of pasteurized process cheese made with different types of emulsifying salts. *Journal of Food Science*, 2008. p. 363-369. DOI: 10.1111/j.1750-3841.2008.00914.x.

[94] SHIRASHOJI, N., JAEGGI, J. J. and LUCEY, J. A. Effects of various emulsifying salts on the rheological and textural properties of pasteurized process Chedar cheese. *Journal of Dairy Science*, 2005, 88. p. 363 – 369. ISSN 0022-0302.

[95] BENNETT, R. J., TRIVEDI, D., HEMAR, Y., REID, D. C. W., ILLINGWORTH, D. and LEE, S. K. The effect of starch addition on the rheological and microstructural properties of model processed cheese. *The Australian Journal of Dairy technology*, 2006, 61, ISSN 0004-9433.

LIST OF ABBREVIATIONS

PC   Processed cheese.

ES   Emulsifying salt.

DM   Dry-matter.

FC   Fat content

DSPP   Disodium hydrogenphosphate

TSPP   Tetrasodium diphosphate

P20   Sodium salt of polyphosphate

TSC   Trisodium citrate

LAB   Lactic acid bacteria

WBC   White-brined cheese

FFA   Free fatty acis

FAA   Free amino acid

LIST OF FIGURES

LIST OF TABLES