TITLE: Rheological and breadmaking properties of wheat flours supplemented with octenyl succinic anhydride-modified waxy maize starches

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Abstract

The feasibility of emulsifying starches as bread improvers was investigated by incorporating starch sodium octenyl succinate (OSA-st), pregelatinized starch sodium octenyl succinate (pregelatinized OSA-st) and hydrolyzed spray-dried starch sodium octenyl succinate (hydrolyzed OSA-st) at 2.5%, 5% and 10% into wheat flour. Dough rheological properties (Mixolab, Alveograph, creep and recovery measurements) and bread quality parameters (specific loaf volume, crust and crumb colour, crumb moisture, crumb grain features, texture) were evaluated. The substituted flours, except hydrolyzed OSA-st, significantly increased water absorption measured by Mixolab. The study on rheological behaviour of doughs containing emulsifying starches, performed by rheometer and Alveograph, showed that OSA-st incorporation yielded strengthened dough, whereas pregelatinized and hydrolyzed OSA-st addition led to more extensible dough. With regards to the thermal behaviour, investigated in water-limited systems by Mixolab, doughs prepared from pregelatinized OSA-st and hydrolyzed OSA-st exhibited lower maximum peak torque, while all three examined starches increased cooking stability and decreased setback value.

Specific volumes of loaves baked from the substituted flours increased, and the highest effect was observed with pregelatinized OSA-st, which consequently produced bread crumbs with the largest mean gas cell area. The bread crumbs baked with octenyl succinate starches were whiter and softer. Although upon one day of storage no significant moisture retention capacity of emulsifying starches was noticed, firmness values of OSA-st and pregelatinized OSA-st supplemented bread crumbs, after 24 h of storage, were similar to or significantly lower than those of control determined 2 h after baking.

The obtained results indicate a requirement for further optimization of the octenyl succinate starch supplemented doughs in terms of combination of different types and levels of modified starches in order to obtain the maximum bread quality.

Key words: OSA modified starch, pregelatinized starch, dough rheology, bread quality

Introduction

Modified starches have been developed in order to enhance the properties of native starch in specific food and non-food applications, such as to improve its water holding capacity, shear, heat, freeze-thaw and low pH resistance, as well as to reinforce its thickening and binding properties, minimize syneresis, etc (Abbas et al., 2010). There are several modification techniques which comprise physical, chemical and enzymatic treatment. Starch can be chemically modified by reactions of conversion (acid-converted starches, oxidized starches and dextrins), cross-linking (distarch phosphate and distarch adipate) and substitution/stabilization (starch esters – starch acetates, starch phosphates and starch succinates; and starch ethers – hydroxypropylated and carboxymethylated starches) (Wurzburg, 2006). Due to their functional benefits in comparison to parent starch, modified starches have already found their application in food manufacturing as fat replacers/mimetics, texture improvers, for high nutritional claim, and for flavor/oil encapsulation (Abbas et al., 2010).

Recent studies have demonstrated that modified starches, blended with wheat flour, can improve bread quality and retard stalling (Miyazaki et al., 2006). According to Miyazaki et al. (2005a, 2005b, 2008) breads prepared with hydroxypropylated tapioca starch had softer bread crumb, firmed at a lower rate and showed a lower endothermic melting enthalpy of amylopectin after three days of storage than those with native tapioca starch or the control sample, whereas addition of phosphorylated cross-linked and acetylated tapioca starches fastened bread staling. Concerning pasting properties, the flour containing phosphorylated cross-linked tapioca starch showed lower peak viscosity and smaller breakdown, whereas that with native, hydroxypropylated and acetylated tapioca starches showed higher peak viscosity and large breakdown than wheat flour (Miyazaki et al., 2005a).

Moreover, Hung & Morita (2004) reported that substitution of wheat flour with 5-15% of cross-linked corn starches and vital wheat gluten resulted in stronger, more stable dough, higher loaf volume and lower crumb firmness after five days of storage in comparison to control.

In order to investigate the role of the starch fraction during breadmaking and storage Goesaert et al. (2008) have incorporated hydroxypropylated and cross-linked modified wheat starches in gluten-starch flour models. Their study revealed that hydroxypropylated starch reduced loaf volume and initial crumb firmness and increased crumb gas cell size, while inclusion of cross-linked starch had little effect on loaf volume or crumb structure but increased crumb firmness. Witczak et al. (2012) and Ziobro et al. (2012) have shown that modified starches (acetylated distarch adipate and hydroxypropyl distarch phosphate) can also be successfully incorporated into gluten-free products in order to improve the rheological properties of dough and quality of bread.

In the studies mentioned above, mostly incorporation of cross-linked, hydroxypropylated, acetylated and phosphorylated types of modified starches was investigated. However, it is possible to chemically modify starch by esterification of native starch with anhydrous octenylsuccinic acid in aqueous suspension at pH 7.0–9.0. Therefore, hydrophobic side chains are introduced to the originally mere hydrophilic starch molecule, meaning that it acquires amphiphilic nature and thus surface-active properties (Tesch et al., 2002). Such emulsifying starch is known as starch sodium octenyl succinate, so called OSA starch and approved by the EU as food additive which goes under the E-number 1450. So far, aqueous solutions of OSA starches have been used in food products to stabilize flavour emulsion in beverages, oil in salad dressings, to encapsulate flavour and as stabilizer and emulsifier in sauces, puddings and baby foods (Dokić et al., 2012). Kim et al. (2001) and Chung et al. (2010) have successfully applied OSA starches as fat replacers in muffins. Recently, it was revealed that OSA-substituted starch reduces postprandial glycemic (Wolf et al., 2001) and insulinemic (Heacock et al., 2004) response relative to a glucose solution, thus indicating that OSA starch has a special nutritional value since it appeared to act as resistant starch (Heacock et al., 2004).

The health benefits of incorporating OSA starches into food products, along with the fact that application of this type of modified starches for breadmaking is rather unknown, stressed the need to investigate the potential of OSA starches as wheat bread improvers. Therefore, the purpose of this study was to assess dough rheological properties and bread quality parameters (volume, colour, crumb moisture, structure and texture) of wheat flour bread containing OSA starches. In addition, the bread quality attributes during storage were also investigated. In order to better understand the role of OSA starches in breadmaking, dual modified (esterified-pregelatinized and esterified-hydrolyzed) starches were also used.

Materials and methods

Materials

Wheat flour (12.9% moisture content, 11.9% protein, 0.64% ash) was provided from the Fidelinka milling company AD, Serbia. The starch sodium octenyl succinates were obtained from waxy maize starch and included:

- Starch sodium octenyl succinate (C*EmTex 06328, Cargill, France)
- Pregelatinized starch sodium octenyl succinate (C*EmTex 12688, Cargill, France)
- Hydrolyzed and spray-dried starch sodium octenyl succinate (C*EmCap 12633, Cargill, France)

All the tested starch sodium octenyl succinates contained less than 3 % octenylsuccinyl groups, which made them food grade starches.

Scanning Electron Microscopy (SEM)

Structure of the starch sodium octenyl succinate samples was analyzed by Jeol JSM 6460LV scanning electron microscope (Tokyo, Japan). Starch samples were mounted on scanning electron microscope (SEM) stubs using double sided adhesive tape and afterwards coated with gold. The ultrastructure of analysed samples was imaged under high vacuum conditions at an accelerating voltage of 25 kV and the obtained micrographs were taken at magnification of ×500.

Evaluation of dough rheological properties

Mixolab measurements

Mixing and pasting behaviour of wheat flour as well as of wheat flour/emulsifying starch mixtures was studied using Mixolab (Chopin Technologies, France), apparatus which simultaneously determines dough rheological characteristics during the process of mixing at constant temperature, as well as during the period of constant heating and cooling (Dapčević Hadnađev et al., 2011). Measurements were performed using the Mixolab "Chopin +" protocol (ICC 173) and the obtained parameters from the recorded curve were: water absorption, Wabs

(%); initial maximum consistency, C1 (Nm) which was used to determine the water absorption; dough development time, DDT (min); stability (min); the minimum torque value at the beginning of heating, C2 (Nm); peak torque or the maximum torque produced during the heating stage, C3 (Nm); cooking stability or the ratio of the torque produced after the period of heating (C4) and the peak torque produced during the heating period (C3), C4/C3; and setback or the difference between the torque produced after cooling at 50°C (C5) and the torque after the period of heating (C4), C5-C4 (Nm).

Alveograph measurements

Dough strength and extensibility of octenyl succinic starch supplemented wheat flours were determined by submitting the dough to a biaxial extension with the use of Chopin Alveograph (Chopin Technologies, France) (Dapčević Hadnađev et al., 2011) and the following parameters were recorded: *W* (10^{-4} J), deformation energy or baking strength; *P* (mm), tenacity or maximum pressure required to reshape the sample; *L* (mm), dough’s extensibility or curve length; *P/L* curve configuration ratio, according to the (ICC 121).

Creep and recovery measurements

Dough samples for creep and recovery measurements were prepared in alveograph mixing bowl following the preparation procedure described in ICC 121. The dough was rested for 10 min in an alveograph mixing bowl.

Creep and recovery measurements were carried out using a Haake Mars rheometer (Thermo Scientific, Germany) equipped with PP35 S serrated parallel plate measuring geometry (35 mm diameter, 1 mm gap) in order to prevent the dough slippage. After loading, the excess of dough sample at the plate edges was neatly trimmed and the edges were sealed with a paraffin oil to prevent the dough from drying during measurements. The dough sample was left to rest for 10 min, so that residual stresses could relax. The measurements were performed at 30±0.1 °C. Creep was recorded at a shear stress of 7 Pa which was within the linear viscoelasticity region for 300 s, followed by a recovery phase of 900 s at a stress of 0 Pa. The parameters obtained were:

maximum creep compliance (J_{max}), relative elastic part of maximum creep compliance (J_e/J_{max}) and relative viscous part of maximum creep compliance (J_v/ J_{max}).

All rheological measurements were performed in triplicates.

Breadmaking Procedure

The basic bread formula, based on flour weight, consisted of: 300 g of the substituted flours (14% mb) in which 0%, 2.5%, 5% or 10% of wheat flour was substituted with the starch sodium octenyl succinates, water up to 500 BU consistency, 2.5% fresh yeast and 2% salt.

Bread doughs were prepared by mixing all ingredients in a 300 g farinograph bowl until they reached maximum consistency plus 1 min. After mixing the dough was fermented in a cabinet at 30 °C for 60 min. Punching down was carried out after 45 min of the fermentation. Then the fermented dough was divided into four pieces (100 g/piece), hand moulded, placed into greased tin pans (90x60 mm in top, 80x50 mm in bottom, and 50 mm in height) and proofed up to the optimum volume increase at 35 °C and the relative humidity of 85% RH for final fermentation. The pieces were baked into a MIWE deck baking oven (Miwe condo, Germany) at 235 °C until the mass loss of 8%. Subsequently, loaves were removed from the pans, cooled for 2 h at room temperature, and then sealed in polyethylene bags to monitor changes in bread quality parameters upon storage (at 22 °C).

Evaluation of bread quality

After cooling to ambient temperature (2 h), the loaves were weighed, and their volume was measured by millet displacement method. Specific volume was calculated as volume/weight (cm^3/g) of four loaves. The moisture of the breadcrumb samples was determined according to ICC 110/1, after 2 and 24 h of storage in three replicates.

Colour measurements

Bread crust and crumb colour measurements were conducted in five replicates per loaf using a Minolta Chroma Meter CR-400 colorimeter (Konica Minolta Sensing Inc., Japan) (8 mm Ø contact area) 2 h after baking. The instrument was calibrated using a standard light white reference tile and the measurements were performed under standard illuminant D65. The obtained results were reported according to the CIELab colour system and they were expressed as the total colour differences (ΔE) between control bread and the samples containing OSA starches.

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

Where ΔL is the lightness difference (L^*=0, black; L^*=100, white), Δa is the redness difference (redness to greenness; positive to negative values, respectively), and Δb is the yellowness difference (yellowness to blueness, positive to negative values, respectively) values.
If $\Delta E < 1$, colour differences are not obvious for the human eye; $1 < \Delta E < 3$, colour differences are not appreciate for the human eye; $\Delta E > 3$; colour differences are obvious for the human eye (Francis & Clydesdale, 1975).

Digital image analysis

Digital image analysis of bread crumb was performed after 24 h of bread storage in polyethylene hermetic bags in which bread was sealed after 2 hours of cooling at room temperature (22ºC). Three bread loaves were sliced transversely using a universal electric slicer (KRUPS 372-75, KRUPS International) to obtain 10-mm thick slices. Two central slices of each loaf were scanned on one side using a flatbed scanner (CanoScan LiDE 100, Canon) with 300 dpi of resolution and supporting scanning software MP Navigator EX. The images were acquired by default settings for brightness and contrast, saved in tiff format and cropped using ImageJ software (National Institutes Health, Bethesda, MD, USA) to obtain the largest possible field of view representing 40 x 50 mm of the slice area. Cropped colour images were converted into an 8-bit greyscale images, while the threshold method used for differentiating gas cells and non-cells was carried out by means of the Otsu algorithm (Gonzales-Barron & Butler, 2006). The obtained crumb features included: the number of cells per square centimetre (cell density), mean cell area (cell size) and cell to total area ratio. The cell was defined as any form larger than 0.01 mm^2, since this area corresponds to a particle with diameter around 0.1 mm which is estimates as the resolving power of human eyes (Archunan, 2004).

Texture measurements

Breadcrumb properties were determined by Texture Profile Analysis (TPA) at room temperature by a TA XT2 Texture Analyser (Stable Micro Systems, England) equipped with a 30-kg load cell and a P/75 – (75 mm diameter) aluminium compression platen. Measurements were performed on five slices (35 mm diameter and 10 mm thickness) taken from the centre of the each loaf. The selected settings were: pre-test 1 mm/s, test speed and post-test speed were 5 mm/s, 75% deformation, and wait time between first and second compression cycle was 5 s.

The breadcrumb samples were compressed twice to give a two bite texture profile curve (Bourne, 2002) and the recorded parameters were hardness, cohesiveness, springiness, chewiness and resilience.

The hardness value was defined as the peak force of the first compression of the bread crumb. Cohesiveness was calculated as a ratio of areas under the curves of the second and first compression, and thus it refers to product resistance to second deformation relative to the first deformation. Springiness is determined as the ratio of the distance measured between the start of the second area and the second probe withdrawal divided by the distance measured between the start of the first area and the first probe withdrawal, and it refers to the crumb elasticity, i.e. ability to return to its original shape after deformation during the first compression. Chewiness is assessed by multiplying hardness, cohesiveness and springiness. Resilience is how well a product "fights to regain its original position". It represents "instant springiness", since resilience is measured on the withdrawal of the first penetration, before the waiting period is started.

Statistical analysis

All analyses were performed in replicates and the mean values with the standard deviations are reported. Analysis of variance and Tukey's multiple range test were performed using Statistica 10.0 (Statsoft, Tulsa, USA). The means were considered significantly different at $p \leq 0.05$.

Results and discussion

Basic characterization of starch sodium octenyl succinates

Chemically modified starches used in this study were starch sodium octenyl succinates obtained from waxy maize starch. However, dual modification preformed on these emulsifying starches gave them different functionality and morphology in comparison to single modified starch which could be noticed in Figure 1. C*EmTex 06328, referred to as OSA-st, is a cook-up starch with emulsifying properties and with remained granular integrity. Conversely, modification performed on C*EmTex 12688, (pregelatinized OSA-st) involved esterification and further pregelatinization (drum drying), whilst C*EmCap 12633 (hydrolized OSA-st) involved enzyme-treatment (hydrolysis) and spray drying. Therefore, the starch granules of hydrolyzed OSA-st were greatly swollen (6 times larger those of OSA-st), but still intact, whereas starch granules of pregelatinized OSA-st were disrupted to a greater extent. Additional modification preformed on pregelatinized OSA-st and hydrolyzed OSA-st made them cold water swellable (Kettlitz et al., 2005; Cargill, 2008a; Cargill, 2008b; Cargill, 2009). Therefore, it was expected that selected three octenyl succinic anhydride modified waxy maize starches would exhibit different impact on wheat flour dough rheology and the quality of final product.

Rheological and thermomechanical properties of wheat flour supplemented with starch sodium octenyl succinates

The effects of starch sodium octenyl succinate incorporation on the thermo-mechanical behaviour of wheat flour are shown in Figure 2 and parameters derived from Mixolab profiles are summarized in Table 1. In general, octenyl succinate starch substituted wheat flours exhibited higher water absorption (Wabs) values than the control. Flour containing hydrolyzed OSA-st was the exception of this trend, since the Wabs was lower in comparison to the control and it decreased with the increase in amount of substitution. The time required for the dough development or time necessary to reach 1.1 Nm consistency (DDT) was significantly affected with the addition of pregelatinized OSA-st and hydrolyzed OSA-st. Control dough was characterized with the presence of two peaks at the consistency of 1.1 Nm. In general, strong wheat flours show a second peak after dough development time, since glutenin, as a predominant fraction of strong flour, will lead to dough stiffening (Weipert, 2006). The addition of 5% and 10% pregelatinized OSA-st and hydrolyzed OSA-st shifted the second peak to lower dough development time values. Moreover, hydrolyzed OSA-st addition decreased the consistency of the first peak due to decreased water absorption, while curves of dough containing pregelatinized OSA-st were characterized by the presence of sharp first peaks during dough development phase, which corresponded to modified starch water absorption. The stability and C2 value as indicators of dough strength, i.e. resistance to mechanical and thermal stresses, respectively, were significantly decreased in doughs containing pregelatinized OSA-st. Addition of hydrolyzed OSA-st also reduced C2 value, while stability was significantly reduced only at 10% substitution.

Water absorptions, as well as the mechanical properties of doughs substituted with emulsifying starches were affected by different factors such as dilution of gluten, characteristics of native starch and types of modification. In general, wheat flour substitution with starches decreases total amount of gluten resulting in formation of weaker protein network (Miyazaki et al., 2006). Miyazaki et al. (2004) reported that decrease in Wabs in dough containing starch hydrolyzates was probably the consequence of flour components dilution, including gluten, damaged starch and pentosans. Moreover, substitution of hydroxyl groups with octenyl succinate groups would impart some hydrophobicity to the hydrophilic starch chain. However, it was revealed that after modification with OSA disruption of the crystalline structure of the starch grains occurs (Sweedman et al., 2013), which could explain slightly higher water absorption of 5 and 10% OSA-st supplemented flours. Further starch granule disruption performed by drum drying, led to amorphous, more open porous structure of the pregelatinized OSA-st (Figure 1), which therefore could absorb more water. Majzoobi et al. (2011) have also shown that pregelatinized starch can absorb more water in comparison to its native counterpart. On the contrary, starch hydrolysis caused decrease in amylopectin molecular weight and thus lowered ability to water absorption of hydrolyzed OSA-st granules. Therefore, inclusion of hydrolyzed OSA-st decreases Wabs, which was in accordance with results obtained by Miyazaki et al. (2004) who also reported decreased water absorption of doughs containing starch hydrolyzates.

During the heating stage, dough containing OSA-st has shown the same pasting properties (C3 value) as the wheat flour dough. On the contrary, wheat flour substituted with pregelatinized OSA-st and hydrolyzed OSA-st showed lower peak torque than wheat flour, where these values decreased with the increase in substitution level. Since in these starches granule integrity was destroyed, they absorbed water during cold stage, while during heating only wheat starch granules swelled. Dilution of wheat starch granules upon addition of starches which are already swollen led to decrease in peak torque. In the systems (e.g. gluten free mixtures with addition of maltodextrin) examined by Witczak et al. (2010) the decrease in peak viscosity was attributed to ability of maltodextrin to limit the amount of water available for starch gelatinization, which retarded amylose leakage from the granule. Moreover, according to Stampfli & Nersten (1995), in bread containing emulsifiers, the adsorption of emulsifiers onto the starch surface might not allow the starch granules to take up water released by gluten to the same extent as the control bread.

Moreover, the dough substituted with cold water swellable starches (pregelatinized OSA-st and hydrolyzed OSA-st) exhibited higher cooking stability (C4/C3 value) than control dough. In addition, hydrolyzed OSA-st expressed no breakdown torque (C4 parameter in Mixolab curve). Generally, waxy starch rapidly develops viscosity but cannot maintain the stability of paste viscosity (Sasaki, 2005). Therefore, Mixolab curves of flours supplemented with higher amount of waxy maize OSA-st expressed low cooking stability. However, they did not express increased peak torque since in Mixolab water limited systems are investigated and therefore there was not enough water for complete granule swelling. Although being waxy starch, pregelatinized OSA-st was characterized with disrupted granule structure which led to increased water absorption during dough cooling to 50 °C and consequently, reduced quantity of free water which resulted in higher paste consistency (C4 torque). On contrary, increase in paste stability of dough containing hydrolyzed OSA-st was not the consequence of increased system viscosity since it reduced water absorption, but probably the result of hydrolyzed OSA-st ability to restrict wheat starch swelling and amylose leaching.

Final torque (C5 parameter in Mixolab curve) of the octenyl succinate starch substituted dough decreased, especially at higher substitution level, indicating that emulsifying starches retards wheat flour gelling. Additionally, flour substituted with octenyl succinate starches generally exhibited greater resistance to retrogradation as indicated by lower setback values (C5-C4 value) in comparison to control. In general, the dramatic setback is the characteristic of starches which contain higher amount of amylose (Thomas & Atwell, 1999). Since emulsifying starches used in this study were waxy maize starches, they contained less than 1 % amylose, and thus their addition reduced the amount of amylose in tested systems.

Hibi (2001) revealed that addition of retrograded waxy corn starch led to decrease in final viscosity. Witczak et al. (2010) also reported lower peak and final viscosities in maltodextrin enriched gluten-free mixtures.

The viscoelastic characteristics of doughs, were further analyzed by creep and recovery tests (Table 2). Under the applied stress of 7 Pa which did not exceed the linear viscoelastic region, dough supplemented with 10% OSA-st exhibited the greatest resistance to deformation as shown by the reduction of maximum creep compliance. On the contrary, addition of 5 and 10% pregelatinized and hydrolyzed OSA-st led to rise in maximum creep compliance values, thus increasing dough extensibility. During the recovery phase, recovered deformation, presented as relative elastic compliance, was the lowest for doughs containing OSA-st, while 5 and 10% pregelatinized OSA-st and hydrolyzed OSA-st showed similar ability to recover deformation after stress removal as control dough. The differences in rheological behaviour of doughs supplemented with different emulsifying starches were the consequence of different starch granule structure. OSA-st granules mostly preserved they crystalline structure (Figure 1) and thus acted as rigid filler which participated in starch and gluten network formation which resulted in stronger doughs. On the contrary, pregelatinized OSA-st granule structure was partially destroyed during thermal and mechanical treatments in drum dryer, while hydrolyzed OSA-st granule was additionally enzymatically treated, which led to amorphous structure and lower polymer molecular weight. These starch polymers dissolved in water during dough preparation and thus increased dough stickiness, which reflected as increase in maximum creep compliance.

Moreover, Alveograph was applied in order to measure dough rheological response to large deformation (Figure 3). Alveograph measurements mostly confirmed results of fundamental rheological test. The strongest dough was obtained by addition of 10% OSA-st which was reflected in higher Alveograph *P* and *W* parameters and low maximum creep compliance in comparison to control sample. On the contrary, inclusion of pregelatinized OSA-st led to increase in dough extensibility (higher maximum creep compliance and Alveograph *L* parameter) and decrease in dough strength (lower *P* and *W* values).

Substitution up to the 5% of hydrolyzed OSA-st did not significantly affected Alveograph W values. However, 10% hydrolyzed OSA-st addition abruptly decreased rheological parameter which refers to dough strength (Alveograph *P* and *W*, and maximum creep compliance) and the obtained dough was soft and sticky.

In general, while addition of OSA-st increased dough strength and resistance to deformation, the addition of pregelatinized OSA-st and hydrolyzed OSA-st increased Alveograph dough extensibility. It was found that these parameters are in correlation with dough rising during proofing and loaf volume. Wang et al. (2002) reported that dough heights decreased for wheat flour samples which expressed decrease in extensibility and increase in resistance measured by Alveograph. According to vanVliet et al. (1992) too high resistance can cause a limited and slow expansion of the gas cells during proofing.

Bread quality parameters of wheat flour supplemented with starch sodium octenyl succinates

Fresh bread quality parameters

The impact of emulsifying starch incorporation on bread crust colour and specific loaf volumes are summarized in Table 3. In general, OSA-st supplemented dough yielded bread with lighter crust and similar specific volume as control bread. However, breads with addition of pregelatinized OSA-st and hydrolyzed OSA-st had darker crusts and higher specific volumes. The values of *ΔE* parameter indicated that colour differences were obvious for the human eye at OSA-st concentration of 10%, and pregelatinized and hydrolyzed OSA-st concentration of 5 and 10%.

The role of gluten and starch in breadmaking has already been well investigated. While the starch gelatinization is the main factor in structuring the bread crumb, the gluten is important for gas retention formed during fermentation and for temporary binding of water required for starch gelatinization (Hibi, 2001). According to Seyhun et al. (2005) different starches have the ability to retain air into cake batter as well as to maintain it during the final stage of baking which result in larger cake volume. These authors have revealed that among different starches (waxy corn, potato starch) incorporation of pregelatinized starch resulted in the highest volume index which was attributed to its property that it is already gelatinized and thus can easily develop cake structure and volume.

Moreover, it was found that waxy starches due to high percentage of amylopectin seem to be more susceptible to α-amylase during fermentation (Lee et al., 2001) and that water-soluble sugars in bread flour such as retrograded waxy corn starch (Hibi, 2001) or high DE value maltodextrin (Witczak et al., 2010) may promote the fermentation of yeast. However, Bao et al. (2003) have revealed that OSA substitution caused a considerable decrease in the extent of degradation of OSA starches by α-amylase, amyloglucosidase, and pullulanase due to the presence of substituents in the starch chains which restrict the activity of the enzymes. In this study, water swellable waxy maize OSA starches gave breads with higher specific volumes and darker bread crusts, while incorporation of non-water soluble OSA starches resulted in non-significantly changed specific volumes and lighter bread crusts in comparison to control bread. Moreover, in contrast to other starch modificates, these starches have emulsifying properties due to the presence of hydrophobic side chains. According to Lazaridou et al. (2007) increased gas retention and better loaf volume of breads containing cellulose modificates could be attributed to their hydrophobic groups which induce additional properties including increased interfacial activity within the dough system during proofing, and forming gel networks on heating during the breadmaking process. Kim et al. (2001) have also found that OSA substitution would increase the air incorporation into muffin dough.

Cross-sectional view of bread crumbs prepared with octenyl succinate starches is presented in Figure 4, while crumb grain features are summarized in Table 4. Concerning crumb structure there were no statistical differences in cell size and density between the control bread and samples containing OSA-st. The number of cells per square centimetre, mean cell area and cell to total area ratio detected in these breads were in accordance with values reported by Goesaert et al. (2008). Addition of pregelatinized OSA-st decreased the number of gas cells detected per square centimetre. Bread crumbs containing pregelatinized OSA-st in concentrations 2.5 and 5% exhibited the largest mean gas cell area. This corresponded to the fact that these breads had the highest specific loaf volumes. Incorporation of hydrolyzed OSA-st affected mean cell area and cell to total area ratio only at the highest examined concentration. This is a great advantage of OSA waxy corn starch in comparison to cross-linked waxy corn starches (Hung & Morita, 2004) or maltodextrins (Witczak et al., 2010) which, although improve softness of bread, produce quite large pores which deteriorate the appearance of bread crumb.

Moreover, an increase in total colour difference, *ΔE* with addition of octenyl succinate starches was observed. This could be seen in Figure 4, and from the results of crumb colour measurements which are summarized in Table 3. Inclusion of octenyl succinate starches gave lighter bread crumbs (higher *L** value), where the highest effect was observed for OSA-st. Kim et al. (2001) also observed the increase in *L** value in cake in produced with octenyl succinate starch was addition. No significant differences in crust redness (positive value of *a** parameter) were noticed among the different emulsifying starches and different substitution levels, while the crumb yellowness (positive value of *b** parameter) was slightly decreased with the addition of cold water swellable OSA starches. However, according to *ΔE* parameter, colour differences were obvious for the human eye only at higher octenyl succinate starches substitution levels.

Stored bread quality parameters

Table 3 also illustrates the changes of bread crumb moisture content for the bread samples containing octenyl succinate starches during 1 day of storage. After cooling to ambient temperature (2 h), breads produced with OSA-st and pregelatinized OSA-st exhibited higher crumb moisture, while breads containing 5 and 10% of hydrolyzed OSA-st expressed lower crumb moisture in comparison to control sample. This was in agreement with the results of Mixolab water absorption. Similar observations were reported by Morita et al. (2002) who found that flour with higher water absorptions resulted in bread crumbs with higher moisture content. Sabanis & Tzia (2011) also noted that breads prepared with higher amount of water exhibited higher crumb moisture values. During storage (24 h), the bread crumb moisture decreased as a consequence of moisture migration from the crumb to the crust thus accelerating starch-gluten interactions and bread firming (He & Hoseney, 1990). However, the water retention capacity of the bread crumbs produced with addition of octenyl succinate starches was mostly decreased in comparison to control bread.

Changes in TPA parameters for the bread samples with octenyl succinate starches during 1 day of storage are presented in Table 5. Incorporation of emulsifying starches decreased initial measured hardness and chewiness. Addition of pregelatinized OSA-st had greatly affected initial hardness, which was decreased by 33-64% in comparison to control sample. Springiness which refer to the crumb elasticity (Bourne, 2002) and cohesiveness were not significantly affected by the type and level of octenyl succinate starch addition. Conversely, resilience of octenyl succinate starch breads which represents "instant springiness", slightly decreased in comparison to control.

Hardness measurements are largely influenced by the volume and density of bread loaves (Goesaert et al., 2008), e.g. the decrease in bread hardness may be a consequence of an increase of total area of gas cells (Skendi et al., 2010), thereby decreasing the force needed to compress the sample. Moreover, softness could be influenced by high moisture content of bread crumbs (Morita et al., 2002). However, there are some studies which indicate that moisture content of bread crumbs is not related to hardness (Hibi, 2001). In this study, pregelatinized OSA-st which had higher specific loaf volume and crumb moisture, has also demonstrated the highest bread crumb softening effect.

Investigations of the causes of bread staling have shown that changes in starch structure, namely, gelatinization and retrogradation contribute to texture from soft to firm (Bloksma & Bushuk, 1988). The additives which compete for water with native wheat starch granules might restrict swelling and solubilisation of the starch during baking, and thus reduce firmness (Gill et al., 2002). Moreover, the reduced crumb hardness of octenyl succinate starch containing breads can be associated with the decreased final and setback torques observed during Mixolab measurements. Addition of emulsifying starches into wheat flour dough presumably reduced the gel forming properties of the amylose polymers, which led to weaker gel structure and, consequently, to a softer bread crumb.

Bread crumb firming is a good indicator of staling, since storage causes an increase in the bread firmness (Seyhun et al., 2005). Hardening during storage is a result of moisture loss as well as starch retrogradation phenomena (Biliaderis et al., 1995). While amylose fraction retrogrades rapidly during initial cooling of bread, slow changes in the amylopectin fraction are responsible for further bread firming (Ghiasi et al., 1984).

During storage, bread crumb hardness increased, while springiness, cohesiveness and resilience decreased (Table 5). Hardness values of OSA-st and pregelatinized OSA-st supplemented bread crumbs, after 24 h of storage, were similar to or significantly lower than those of control determined after baking. However, while firming rate of pregelatinized OSA-st containing bread was similar to that of control, OSA-st incorporated in concentration of 5% and higher demonstrated lower firmness increase.

Hung et al. (2006) reported that incorporation of waxy wheat starch resulted in retaining more moisture in breadcrumbs and retardation of the staling. Softening effect of hydrocolloids and dietary fibers is also attributed to their high water retention capacity, and possible inhibition of amylopectin retrogradation (Biliaderis et al., 1995; Lazaridou et al., 2007). The role of emulsifiers as crumb-softening agents is related to their interaction with starch, particularly formation of inclusion complex with amylose, which does not participate in gel formation during baking (Stampfli & Nersten, 1995).

However, since octenyl succinate starches did not exhibit high water retention capacities, their softening effect could be attributed to weakening of wheat starch network structure, which was manifested as decrease in Mixolab setback value. Moreover, Thirathumthavorn & Charoenrein (2006) have suggested the possibility of formation of amylose–OSA inclusion complexes.

Conclusions

The impact of modification type and amount of octenyl succinate starches on wheat flour dough and bread quality characteristics was investigated. Rheological properties of dough studied by Mixolab, Alveograph and creep measurements showed that: 1) incorporation of cook up emulsifying starch (OSA-st) resulted in an increase in water absorption and dough resistance to deformation; 2) addition of pregelatinized OSA-st starch also influenced a rise in water absorption values while dough strength was decreased and extensibility increased and 3) substitution of hydrolyzed OSA-st starch led to decrease in Wabs parameter and dough resistance to deformation. On heating, OSA-st containing dough has shown the same maximum peak consistency as wheat flour dough, while pregelatinized OSA-st and hydrolyzed OSA-st lowered maximum peak consistency. Additionally, doughs enriched with octenyl succinate starches mostly exhibited greater resistance to retrogradation as indicated by lower Mixolab setback values in comparison to control.

Addition of emulsifying starches in wheat flour dough contributed to breads with higher loaf volume which was especially noticed in samples containing cold water swellable starches which also increased dough extensibility. Higher crumb softness of octenyl succinate containing breads compared to control bread was presumably due to reduction of the gel forming properties of the amylose polymers, which was revealed by decrease in setback values. Pregelatinized OSA-st which yielded bread crumb with the softest texture was also characterized with the highest crumb moisture, mean cell area and bread loaf volume. Moreover, breads containing octenyl succinate starches had whiter bread crumbs in comparison to control bread crumb.

Although upon 24 h of storage no significant increase in moisture retention capacity or decrease in firming rate of bread crumb prepared with octenyl succinate starches were noticed; firmness values of OSA-st and pregelatinized OSA-st supplemented bread crumbs were similar to or significantly lower than those of control determined after baking. However, further studies have to be performed in order to reveal octenyl succinate starch supplemented breads stalling kinetics, as well as to optimize the combination of different types and levels of octenyl succinate starches which will result in maximum bread quality.

In general, the results demonstrated that emulsifying starches could be used in breadmaking in order to improve the quality of bread.

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Figure captions

Figure 1. Scanning electron micrographs, at 500 magnifications, of a) starch sodium octenyl succinate, b) pregelatinized starch sodium octenyl succinate and c) hydrolysed and spray dried starch sodium octenyl succinate

Figure 2. Mixolab profiles of starch sodium octenyl succinate substituted wheat flour doughs

Figure 3. Alveograph parameters of starch sodium octenyl succinate substituted wheat flour doughs

Figure 4. Internal structure of starch sodium octenyl succinate substituted wheat flour breads; A) control, B) 10% OSA-st, C) 10% pregelatinized OSA-st and D) 10% hydrolyzed OSA-st

Table 1. Mixolab parameters of starch sodium octenyl succinate substituted wheat flour doughs(a)

Sample	Wabs (%)	DDT (min)	Stability (min)	C2 (Nm)	C3 (Nm)	C4/C3	C5-C4 (Nm)
Control	55.4±0.14^{d}	5.26±0.04^{cd}	9.70±0.18^{c}	0.45±0.01^{g}	1.78±0.01^{f}	0.89±0.01^{cd}	0.75±0.01^{d}
OSA-st							
2.5%	55.7±0.14^{de}	4.22±0.26^{b}	9.24±0.16^{c}	0.45±0.01^{fg}	1.79±0.01^{f}	0.89±0.01^{cde}	0.76±0.05^{d}
5%	56.0±0.14^{e}	4.64±0.11^{ac}	9.47±0.25^{c}	0.43±0.01^{ef}	1.73±0.01^{ef}	0.80±0.01^{b}	0.61±0.04^{bc}
10%	56.1±0.07^{e}	4.96±0.09^{cd}	9.31±1.07^{c}	0.44±0.01^{efg}	1.76±0.03^{f}	0.76±0.01^{a}	0.60±0.01^{bc}
Pregelatinized OSA-st							
2.5%	58.1±0.07^{f}	4.24±0.40^{b}	6.69±0.23^{b}	0.32±0.01^{c}	1.50±0.01^{d}	0.91±0.01^{def}	0.43±0.01^{a}
5%	59.6±0.14^{h}	3.11±0.08^{a}	4.43±0.06^{a}	0.27±0.01^{b}	1.40±0.02^{c}	0.87±0.01^{c}	0.60±0.03^{bc}
10%	59.0±0.07^{g}	3.12±0.02^{a}	4.70±0.21^{a}	0.21±0.01^{a}	1.15±0.01^{a}	0.94±0.01^{fg}	0.52±0.01^{ab}
Hydrolyzed OSA-st							
2.5%	54.6±0.21^{c}	6.39±0.01^{e}	9.20±0.03^{c}	0.42±0.01^{e}	1.67±0.03^{e}	0.93±0.01^{efg}	0.76±0.03^{d}
5%	53.8±0.21^{b}	5.63±0.16^{d}	9.12±0.19^{c}	0.35±0.01^{d}	1.54±0.02^{d}	0.96±0.01^{g}	0.63±0.04^{bc}
10%	52.3±0.07^{a}	4.00±0.14^{b}	4.65±0.07^{a}	0.25±0.01^{b}	1.28±0.01^{b}	1.07±0.01^{h}	0.68±0.01^{cd}

(a) Mean value ± standard deviation of three replicates; values followed by the same letter in the column are not significantly different ($p > 0.05$)

Table 2. Creep and recovery parameters of starch sodium octenyl succinate substituted wheat flour doughs[(a)]

Sample	Max. creep compliance, J_{max} (10^{-5} Pa^{-1})	Relative elastic part of J_{max}, J_e / J_{max} (%)	Relative viscous part of J_{max}, J_v / J_{max} (%)
Control	76.2±1.3^{bc}	69.7±3.8^{e}	30.3±3.8^{a}
OSA-st			
2.5%	74.5±3.2^{abc}	47.9±3.6^{abc}	52.1±3.6^{cde}
5%	73.8±1.3^{ab}	46.6±1.7^{ab}	53.4±1.7^{de}
10%	52.7±0.8^{a}	44.4±2.8^{a}	55.6±2.8^{e}
Pregelatinized-st OSA			
2.5%	96.8±4.3^{cd}	56.0±4.2^{bcd}	43.9±4.2^{bcd}
5%	109.8±5.7^{de}	63.8±1.2^{de}	36.2±1.2^{ab}
10%	113.2±10.6^{e}	70.2±2.0^{e}	29.8±2.0^{a}
Hydrolyzed OSA-st			
2.5%	96.7±6.2^{bcd}	57.3±1.5^{cd}	42.7±1.5^{bc}
5%	101.9±7.6^{d}	61.0±1.5^{de}	39.0±1.5^{ab}
10%	165.9±8.1^{f}	60.7±2.3^{de}	39.3±2.3^{ab}

[(a)] Mean value ± standard deviation of three replicates; values followed by the same letter in the column are not significantly different ($p > 0.05$)

Table 3. Specific volume, crust and crumb colour, and crumb moisture during storage of starch sodium octenyl succinate substituted wheat flour breads(a)

Sample	Specific volume (cm^3/g)	ΔE crust	ΔE crumb	Crumb moisture (%) 2 h	Crumb moisture (%) 24 h
Control	2.41 ± 0.01^{a}	-	-	$41.95\pm0.04^{c,2}$	$41.32\pm0.03^{e,1}$
OSA-st					
2.5%	2.48 ± 0.03^{a}	1.71±0.16	3.64±0.29	$42.46\pm0.02^{d,2}$	$40.81\pm0.01^{d,1}$
5%	2.46 ± 0.01^{a}	1.61±0.15	3.78±0.31	$42.40\pm0.06^{d,2}$	$40.87\pm0.02^{d,1}$
10%	2.50 ± 0.03^{a}	6.58±0.26	5.07±0.72	$42.40\pm0.10^{d,2}$	$41.81\pm0.01^{g,1}$
Pregelatinized OSA-st					
2.5%	2.69 ± 0.07^{b}	2.05±0.26	1.02±0.21	$42.36\pm0.03^{d,2}$	$41.69\pm0.04^{fg,1}$
5%	2.84 ± 0.07^{c}	6.10±0.76	4.09±0.63	$42.82\pm0.01^{e,2}$	$41.60\pm0.03^{f,1}$
10%	2.80 ± 0.07^{c}	3.86±0.29	6.14±0.44	$43.81\pm0.17^{f,2}$	$41.24\pm0.05^{e,1}$
Hydrolyzed OSA-st					
2.5%	2.46 ± 0.01^{a}	1.24±0.15	1.29±0.21	$42.30\pm0.01^{d,2}$	$40.37\pm0.05^{c,1}$
5%	2.68 ± 0.01^{b}	3.94±0.44	2.02±0.36	$41.56\pm0.01^{b,2}$	$40.12\pm0.02^{b,1}$
10%	2.72 ± 0.01^{b}	6.49±0.27	4.78±0.24	$41.19\pm0.04^{a,2}$	$38.10\pm0.03^{a,1}$

(a) Mean value ± standard deviation; values followed by the same letter in the column or number in the row are not significantly different ($p > 0.05$)

Table 4. Computed bread crumb features of starch sodium octenyl succinate substituted wheat flour breads$^{(a)}$

Sample	Cells/cm^2	Mean cell area (mm^2)	Cell/total area ratio (%)
Control	88±7^{cd}	0.45±0.03^{ab}	39.0±1.3^{ab}
OSA-st			
2.5%	82±5^{bcd}	0.46±0.03^{ab}	37.6±1.6^{a}
5%	76±7^{b}	0.50±0.04^{bc}	37.8±1.5^{a}
10%	88±4^{d}	0.41±0.03^{a}	36.6±1.6^{a}
Pregelatinized OSA-st			
2.5%	64±2^{a}	0.63±0.04^{d}	40.7±1.7^{abc}
5%	64±4^{a}	0.67±0.04^{d}	42.6±0.7^{bc}
10%	78±3^{bc}	0.48±0.04^{abc}	37.1±3.1^{a}
Hydrolyzed OSA-st			
2.5%	84±4^{bcd}	0.46±0.03^{abc}	38.7±1.3^{ab}
5%	85±8^{bcd}	0.44±0.05^{ab}	37.2±2.6^{a}
10%	83±2^{bcd}	0.54±0.05^{c}	44.7±3.1^{c}

(a) Mean value ± standard deviation; values followed by the same letter in the column are not significantly different ($p > 0.05$)

Table 5. Texture profile analysis of starch sodium octenyl succinate substituted wheat flour bread crumbs$^{(a)}$

Sample	Hardness (g)		Springiness		Cohesiveness		Chewiness (g)		Resilience	
	2 h	24 h	2 h	24 h	2 h	24 h	2 h	24 h	2 h	24 h
Control	$14910\pm1792^{c,1}$	$18588\pm359^{e,2}$	$0.97\pm0.01^{a,2}$	$0.93\pm0.01^{bc,1}$	$0.75\pm0.01^{abc,2}$	$0.63\pm0.01^{bcd,1}$	$10891\pm1383^{c,1}$	$10798\pm466^{f,1}$	$0.46\pm0.02^{b,2}$	$0.33\pm0.01^{bc,1}$
OSA-st										
2.5%	$11302\pm674^{b,1}$	$15098\pm1310^{cd,2}$	$0.99\pm0.02^{a,2}$	$0.94\pm0.01^{bc,1}$	$0.75\pm0.01^{abc,2}$	$0.63\pm0.02^{cd,1}$	$8447\pm424^{b,1}$	$8937\pm924^{cde,1}$	$0.41\pm0.01^{a,2}$	$0.31\pm0.02^{abc,1}$
5%	$10865\pm905^{b,1}$	$13519\pm1750^{bc,2}$	$0.98\pm0.02^{a,2}$	$0.93\pm0.02^{bc,1}$	$0.77\pm0.03^{c,2}$	$0.60\pm0.01^{ab,1}$	$8199\pm873^{b,1}$	$7507\pm1091^{abc,1}$	$0.43\pm0.02^{ab,2}$	$0.28\pm0.02^{a,1}$
10%	$9879\pm1106^{b,1}$	$11469\pm774^{ab,2}$	$0.99\pm0.01^{a,2}$	$0.96\pm0.01^{c,1}$	$0.75\pm0.01^{abc,2}$	$0.63\pm0.01^{cd,1}$	$7332\pm749^{b,1}$	$6936\pm510^{ab,1}$	$0.40\pm0.02^{a,2}$	$0.30\pm0.02^{abc,1}$
Pregelatinized OSA-st										
2.5%	$10054\pm957^{b,1}$	$14074\pm1510^{c,2}$	$0.97\pm0.02^{a,2}$	$0.94\pm0.02^{bc,1}$	$0.75\pm0.01^{abc,2}$	$0.61\pm0.01^{abcd,1}$	$7274\pm597^{b,1}$	$8105\pm834^{bcde,1}$	$0.41\pm0.01^{a,2}$	$0.30\pm0.01^{abc,1}$
5%	$6604\pm270^{a,1}$	$9467\pm855^{a,2}$	$1.01\pm0.01^{a,2}$	$0.96\pm0.02^{c,1}$	$0.77\pm0.00^{bc,2}$	$0.64\pm0.01^{d,1}$	$5093\pm168^{a,1}$	$5770\pm695^{a,1}$	$0.39\pm0.01^{a,2}$	$0.32\pm0.02^{abc,1}$
10%	$5433\pm659^{a,1}$	$10024\pm1136^{a,2}$	$1.00\pm0.02^{a,2}$	$0.96\pm0.01^{c,1}$	$0.77\pm0.01^{c,2}$	$0.63\pm0.02^{cd,1}$	$4242\pm414^{a,1}$	$5995\pm705^{a,2}$	$0.41\pm0.03^{a,2}$	$0.33\pm0.02^{c,1}$
Hydrolyzed OSA-st										
2.5%	$14620\pm1099^{c,1}$	$17530\pm1460^{de,2}$	$0.97\pm0.02^{a,2}$	$0.91\pm0.02^{ab,1}$	$0.73\pm0.01^{a,2}$	$0.59\pm0.02^{a,1}$	$10373\pm860^{c,1}$	$9541\pm1156^{ef,1}$	$0.43\pm0.02^{ab,2}$	$0.29\pm0.02^{ab,1}$
5%	$11035\pm742^{b,1}$	$17635\pm1259^{de,2}$	$0.98\pm0.02^{a,2}$	$0.88\pm0.01^{a,1}$	$0.74\pm0.01^{ab,2}$	$0.61\pm0.02^{abc,1}$	$7973\pm446^{b,1}$	$9454\pm792^{def,2}$	$0.40\pm0.01^{a,2}$	$0.31\pm0.02^{abc,1}$
10%	$10065\pm1442^{b,1}$	$13774\pm1045^{bc,2}$	$0.98\pm0.02^{a,1}$	$0.94\pm0.03^{bc,1}$	$0.75\pm0.01^{abc,2}$	$0.58\pm0.01^{a,1}$	$7383\pm1130^{b,1}$	$7483\pm719^{abcd,1}$	$0.40\pm0.03^{a,2}$	$0.28\pm0.01^{a,1}$

$^{(a)}$ Mean value ± standard deviation of five replicates; values followed by the same letter in the column or number in the row within the same parameter are not significantly different ($p > 0.05$)

Figure 1

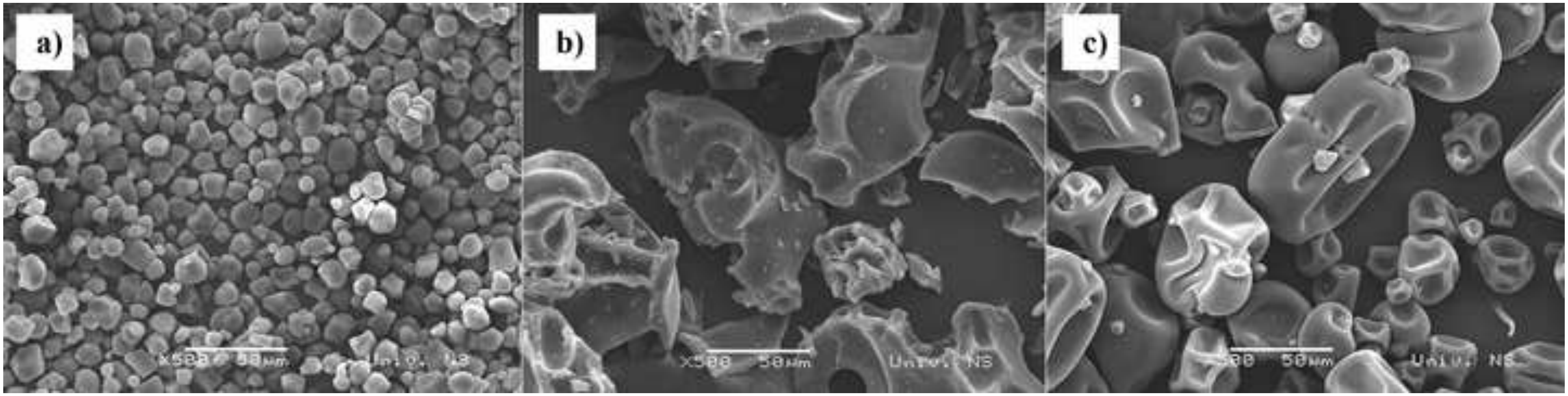

Figure 2

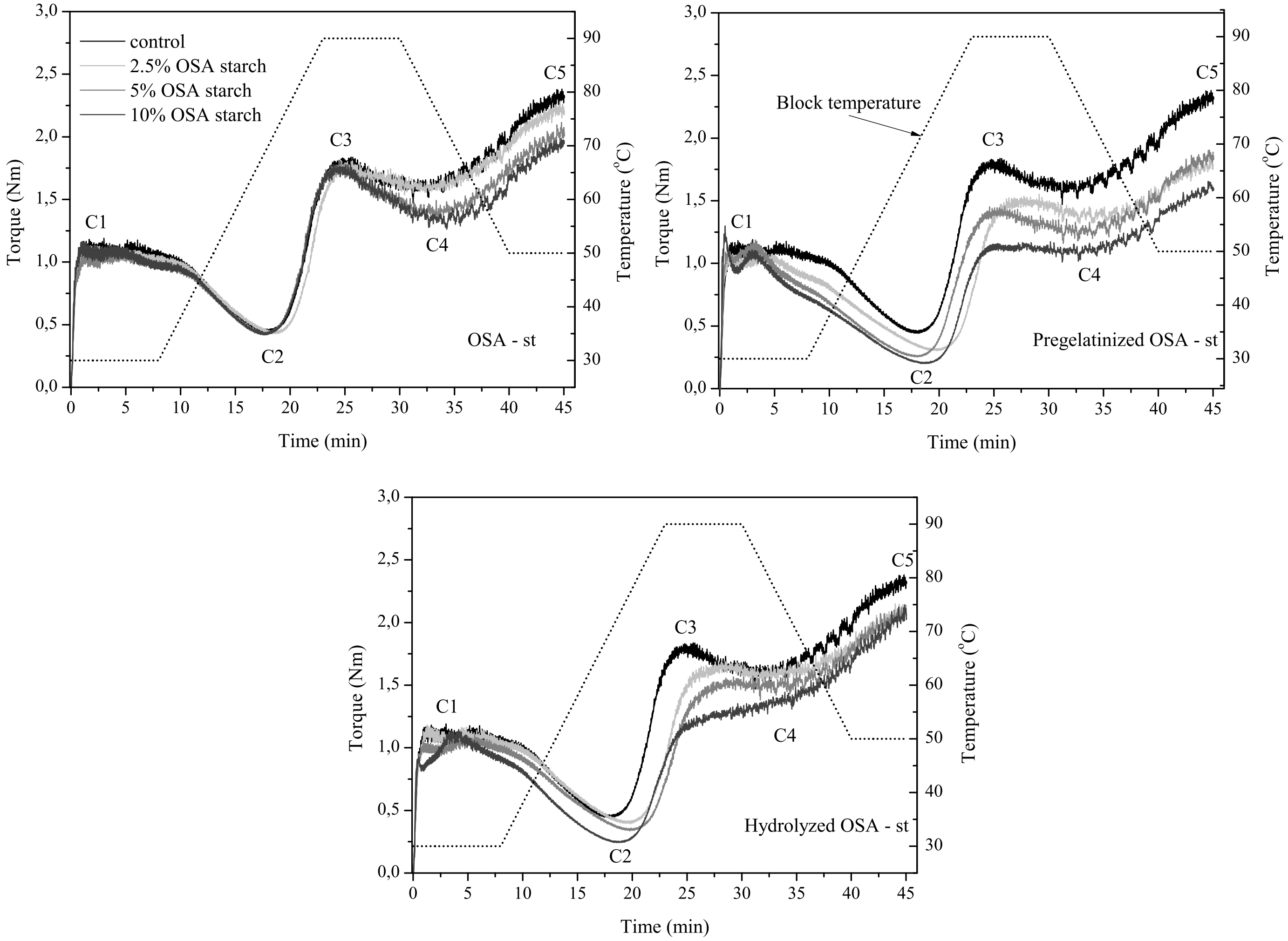
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Torque (Nm)
control
2.5% OSA starch
5% OSA starch
10% OSA starch
C1
C2
C3
C4
C5
90
80
70
60
50
40
30
Temperature (°C)
OSA - st
0
5
10
15
20
25
30
35
40
45
Time (min)
Block temperature
Pregelatinized OSA - st
Hydrolyzed OSA - st

Figure 3

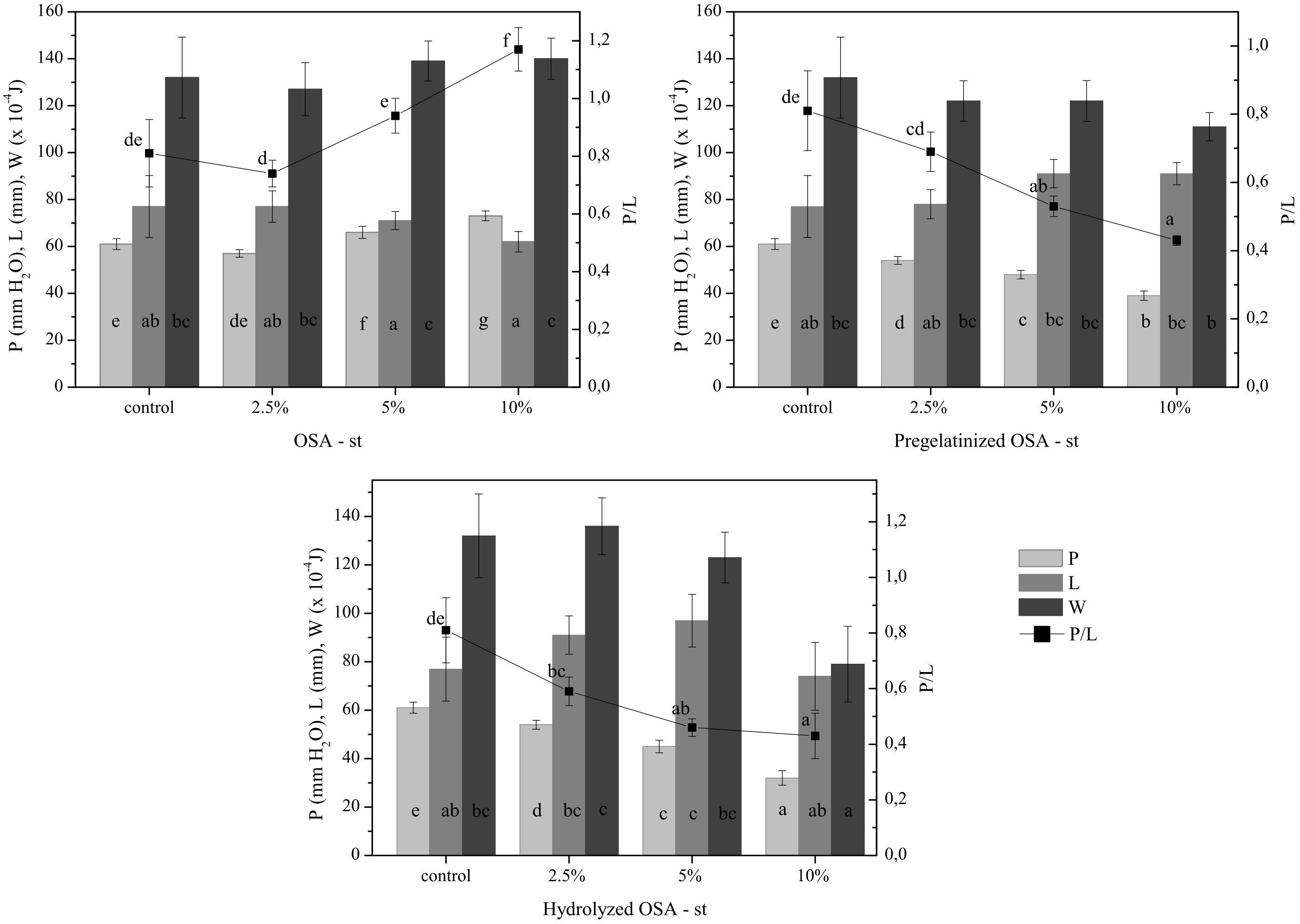
P (mm H_2O), L (mm), W (x 10^{-4}J)
P/L
160
140
120
100
80
60
40
20
0
0,0
0,2
0,4
0,6
0,8
1,0
1,2
control
2.5%
5%
10%
OSA - st
de
d
e
f
e ab bc
de ab bc
f a c
g a c
Pregelatinized OSA - st
de
cd
ab
a
e ab bc
d ab bc
c bc bc
b bc b
Hydrolyzed OSA - st
de
bc
ab
a
e ab bc
d bc c
c c bc
a ab a
P
L
W
P/L

Figure 4