The Effect of Enzyme Addition on the rheological Characteristics of the Gluten-free Dough and Bread

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Doctoral Thesis Summary

Tomas Bata University in Zlín

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Faculty of Technology

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Vliv přídavku enzymu na reologické vlastnosti bezlepkového těsta a pečiva

The Effect of Enzyme Addition on the rheological Characteristics of the Gluten-free Dough and Bread

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SUMMARY

The Doctoral Thesis deals with the issue of rheological properties of Gluten-free different types of rice flour and effect of the α- amylase enzyme on the rheological characteristics of the gluten-free flour dough and bread. The purpose of this study is to discuss the significant role of the α-amylase enzyme in the production and rheological characteristics of gluten-free rice dough and baked bread. There were different types of gluten-free rice flours tested in this study, including rice fine flour, semi-coarse, red rice flour, rice flour with 0.5S, extra-fine rice flour, white sticky rice flour, and black rice flour. We performed small and large deformation methods to evaluate how the addition of the α-amylase enzyme affected the rheological characteristics of dough and the final product (bread). The α-amylase enzyme has been used as an additive and the rheology (small-deformation oscillation and long deformation) properties have been determined. Specific volume, baking loss, and Texture profile analysis (TPA) parameters were used to evaluate the baking properties of bread. Microstructural changes were also observed following the addition of enzymes to doughs. In the course of small deformation studies of doughs, the doughs containing 0.5S and extra-fine flour showed the most significant changes after enzyme addition. 0.5S flour dough was less resistant to extension, area, and extensibility after the addition of α-amylase. There was no significant difference in extensibility for dough made with extra-fine flour. As a function of α-amylase, significant changes were observed in the flour doughs' properties during the heating process. The G' (storage modulus), G'' (loss modulus), and η' (viscosity) values of the 0.5S and extra-fine doughs decreased with the addition of α-amylase. Following the enzyme addition, $G'<G''$ was only observed in 0.5S rice flour dough. Temperature-dependent changes were observed. The rheology of the samples depended on the quality of the baking material used. The presence of the α- amylase enzyme affected the bread's texture. In microstructure results the controldough samples; the starch granule microstructures were complex and not easily visible. It can be assumed that α- amylase enzyme can be used to improve the quality of rice flour dough and breads quality.

Abstraktní

Disertační práce se zabývá problematikou reologických vlastností různých typů bezlepkové rýžové mouky a vlivu enzymu α-amylázy na reologické vlastnosti těsta a chleba z bezlepkové mouky. Cílem této studie je diskutovat významnou roli enzymu α-amylázy v procesu výroby a reologických vlastnostech bezlepkového rýžového těsta a chleba. V této studii byly testovány různé druhy bezlepkových rýžových mouk, včetně rýžové mouky jemné, polohrubé, mouky z červené rýže, rýžové mouky s obsahem 0,5S, jemnou rýžovou mouku, bílou lepkavou rýžovou mouku a černou rýžovou mouku. Byly použity malé a velkou deformační metodu, pro vyhodnocení, jak přídavek α-amylázy ovlivnil reologické vlastnosti těsta a výsledného výrobku (chleba). Enzym α-amyláza byl použit jakoaditivum a reologická charakteristika (oscilace při malé deformaci a dlouhá deformace) byla determinována. Specifický objem, ztráty pečením an analýza profilu textury (TPA) byly použity k hodnocení pekařských vlastností chleba. Mikrostrukturální změny byly rovněž pozorovány po přidání enzymů do těsta. Během studií malých deformací těst byla těsta s obsahem 0,5S an extra jemnou mouku vykazovala po přídavku enzymůnejvýraznější změny. Těstoz 0.5S mouky bylo méně odolné vůči roztažení a roztažitelnosti popřidání α- amylázy. Nebyl zjištěn žádný významný rozdíl v roztažnosti pro těsta vyrobená zextra jemné mouky. V závislosti na přídavku α-amylázy byly pozorovány změny ve vlastnostech těst během zahřívání. Hodnoty *G*’ (modul skladovatelnosti), *G*” (ztrátový modul) a *η*’ (viskozita) se snížily s přídavkem α- amylázy u 0,5S an extra jemných těst. Popřídavku enzymu bylozvýšení *G’*< *G*” pozorováno pouze u rýžového těsta 0,5S. Byly pozorovány změny závislé na teplotě. Reologie vzorků závisela na kvalitě použitého pekařského materiálu. Přítomnost enzymu α-amylázy ovlivnila texturu chleba. Ve výsledcích mikrostruktury kontrolních vzorků těsta byla mikrostruktura škrobových granulí složitá a špatně viditelná. Lze předpokládat, že enzym α-amyláza může být použit ke zlepšení kvality těsta z rýžové mouky a kvality chleba.

CONTENT

INTRODUCTION

The rheological properties of the dough are dependent both on the quantity and quality of gluten proteins. In addition to retaining fermentation gas, the gluten network can influence the stability of gas cells during expansion, contribute to the characteristics of a soft (spongy) and elastic crumb, and influence the appearance of wheat-based bread and baked goods [1]. A gluten's extensibility is determined by the gliadin fraction, while its elasticity is determined by glutenin. Gluten exhibits these physical properties that are responsible for the unique viscoelastic characteristics of wheat dough and the quality of wheat-based bread and baked products [2].

Rice flour is usually preferred in GF bread-making because of its colorlessness, nutritional characteristics, bland taste, and low hypoallergenic properties. Rice, corn, sorghum, and buckwheat are the main gluten-free cereals which are recommended for celiac patients. Except for rice, at a concentration of 10-20%, corn, buckwheat, and sorghum affected the product quality [3]. The presence of low-level of sodium and easily digested carbohydrates makes rice an ideal food for celiac disease patients [4]. However, despite the numerous advantages of rice flour, the lack of gluten proteins makes it very difficult to obtain an acceptable yeast-leavened product such as bread because of the absence of a proper network necessary to hold the carbon dioxide produced during proofing. The absence of gluten in bread making brings technological problems [5]. Compared to the wheat dough, the extensibility of gluten-free doughs, as well as the ability to stretch, is lower. GF dough viscosity during baking is not optimal. Different approaches have been presented to overcome that problem; xanthan gum and carboxymethylcellulose have been used as gluten substitutes for preparing bread without gluten [6] – [8]. Protein cross-linking, or the formation of covalent bonds between polypeptide chains, is a means of modifying the protein functionality and simultaneously broadening its applications in different processes [9], [10]. In the recent past, enzyme have been successfully applied in several GF food systems for their unique ability to modify protein functionality and to promote protein cross-linking. Further, the enzymes are more favored due to increasing consumer awareness about the use of chemicals in foods [6], [11].

1. THE CURRENT STATE RESEARCH

1.1 Problems with Gluten-free

Today's GF bakery products have lower palatability than their conventional counterparts and may cause vitamin, mineral, and fiber deficiencies. The usage of gluten-free flour results in bread with a crumbling texture, poor color, and other flaws [12]. Without gluten, maintain good sensory quality, structure, and retention of softness during storage is a big challenge in the baking application [13].

Problems related to volume and crumb texture are associated with GF bread even when rice flour is used, which seems to be the best raw material for this type of bread [14]. The unattractive appearance of GF bread still remains a challenge in the GF bread-making. In recent years, enzyme (transglutaminase and cyclodextrinase) and hydrocolloids (carboxymethylcellulose and hydroxypropylmethylcellulose) have become the main focus for the improvement of GF bread [9]. Transglutaminase has been shown to improve the dough viscoelasticity and decrease the crumb hardness of the bread. Cyclodextrinase also enhances dough viscoelasticity.

1.2 Gluten-free flours and additives

GF flour such as rice, rye, corn, oats, buckwheat, and blends consist of various components. The starch and protein components are essential for the transformation of a GF dough foam-type system to a bread-like system [11]. There are several types of GF flours, so it can be a challenge to decide which flour is best for the different types of baking. Flours that are made from seeds (amaranth, buckwheat, rice, corn, millet, sorghum), buy them labeled gluten-free since many are grown and processed in facilities that also manufacture wheat, barley, or rye and cross-contact can occur. The needs for the development of good tasting and healthy GF products are due to consumer awareness about gluten allergenicity and CD [15]. Many ingredients and improvers were used in bread manufacturing to overcome the problems associated with rice flour. Rice flour, corn starch, and cassava starch mixture are used for the optimization of the gluten-free bread [16].

1.3 Rice flour

The staple food for the Asian countries is rice. The worldwide production rate of the rice is the second of the third highest after wheat [17]. A small number of prolamins (2.5-3.5%) are present in rice. It can be used with other flours as a combination and individual.

1.4 Hydrocolloids

Hydrocolloids interact with water and produce a gel network structure that impacts dough viscosity and strengthens the dough film surrounding the expanding cells, increasing gas retention capability during proofing and baking, and enhancing the volume, structure, texture, and appearance of gluten-free bread [18]. Hydrocolloidsalso have a "water-release" effect necessary for starch gelatinization during baking.

1.5 Proteins

Proteins are used in GF formulations with the dual objective of enhancing both the sensory and the nutritional (increasing protein content and supplying essential amino acids) properties of gluten-free bread. They are mainly used to build up a network that can mimic some of the gluten's properties, improving the dough's rheological and baking properties, along with the structural, sensory, and shelf-life characteristics of gluten-free bread [14, 43].

1.6 The factors affecting enzymes activity

- Type of substrate and concentration: protein, carbohydrate, fat.
- Enzyme specificity
- Enzyme amount
- pH: acid, neutral, basic
- Time of reaction
- Temperature
- Inhibitors: heating temperature [52, 53]

2. Amylase

Amylases are amylolytic enzymes that break down starches into sugars and are very widespread throughout the animal, plant, and microorganism. Various amylases exist which cleave the branches of starch molecules with a particular specificity. Certain amylases can delay crumb firming and hence function as anti-staling agents. Possible mechanisms or modes of action whereby these enzymes retard the staling/firming process are discussed below. Typical amylase-containing anti-staling products mainly consist of bacterial or fungal α-amylases with intermediate thermostability [23].

2.1 α-Amylase

α-Amylases are enzymes that catalyze the hydrolysis of internal α-1,4- glycosidic linkages in polysaccharides into low-molecular-weight products, such as glucose, maltose, and maltotriose units [24]. α -amylases act on starch, glycogen, and related polysaccharides in a random manner; reducing groups are liberated in the α-configuration. α-1,4- glucan-4-glucanohydrolase is the scientific name of α-amylase. It is secreted by animals, plants, and microorganisms [25].

2.3 Effect of α-Amylase on dough and bread rheology

α-amylases are usually added to optimize the amylase activity of the flour, initially aiming to increase the levels of fermentable and reducing sugars. The supplemented α-amylases break down damaged starch particles into low molecular weight dextrins during the dough stage [26], [27]. In addition, these enzymes can improve the gas-retention properties of fermented dough and reduce dough viscosity during starch gelatinization, with consequent improvements in product volume and softness [23], [28].

3. AIMS OF THE RESEARCH

The aims of this study were as follows.

i. To determine the impact of α-amylase enzyme on the rheological properties (small-deformation & long deformation) of different types of rice flour doughs.
ii. To determine the microstructural changes in the dough starch molecules after α-amylase enzyme addition.
iii. To determine the impact of the added α-amylase enzyme on the textural characteristics of rice bread.
iv. To evaluate the impact of α-amylase enzyme on loaf specific volume and baking loss).

4. MATERIAL AND METHOD

4.1 Material

Different types of rice flour (fine flour, semi-coarse, red, white sticky rice, black, red, and extra-fine, manufactured by FLOUR MEDICAL spol s.r.o, Brno, Czech Republic ADVENI, while 0.5 S flour produced by Extrudo a.s. Týn nad Vltavou, Czech Republic) were involved in this study. The fungal enzyme (α-amylase) originated by *Aspergillus orygal var* was provided by DANISCO Edwin Rahrs Vej DK.

4.2 Dough formulation

A formula for dough preparation consisted of rice flour (100 %), water (90 %), sucrose (1.86 %), salt (1.50 %), dry yeast (1.80 %), and α- amylase enzyme. The amount of enzyme was added according to the falling number of the flour, enzyme amount added in the flour until the falling number was decreased to 62 s. The amounts of the ingredients were related to 100 g of flour dry matter. Dry yeast was reactivated for 10 ± 1 min. in a sugar solution (35 ± 1 °C). The dough ingredients were placed into an Eta gratus mixer bowl (Eta a. s. Czech Republic) and mixed for 6 min.

4.3 Moisture content determination

The sample of flour (5 g) was weighed and placed in the moisture meter. The sample was heated at 130 °C in the moisture meter (temperature heating). Moisture content was determined by heating the flour in a moisture meter (OHAUS, MB120). Each sample was tested twice

4.4 Hagberg falling number determination

It was determined that the amount of flour sample taken was dependent on the moisture content of the flour. The test was conducted using the Perten Hagberg instrument. Each sample was tested twice.

4.5 Uniaxial elongation test

The gluten-free dough resistance to extension, extensibility, and other characteristics can be obtained by uniaxial dough deformation. The doughs samples were prepared according to the dough preparation section (4.2.1), but for the rheological tests the dough preparation was without yeast addition. Test samples were with a trapezoidal cross-section (3mm, 5mm, 4mm) with 5 cm long pieces. The tests were performed using textural analyzer TA. XT plus (Stable micro system

Ltd., UK) equipped with an SMS/Kieffer dough and gluten extensibility Rig. During the testing, the sample was stretched by hook until it fractured. The test speed of the hook was 3.00 mm/s and trigger force 5 g. The required force to stretch dough sample and displacement the hook recorded as a function of time [29]. Each sample was tested in 6X.

4.6 Dynamic oscillation test

The effect of enzyme addition on the rheological characteristics of dough is studied at 30 - 90 °C temperature ramp simulating dough baking. Rheological measurements were performed using HAAKE Rheo Stress 1 (Thermo Scientific, Czech Republic). The dough samples were prepared manually according to the dough preparation method (see section 4.2.1), excluding dry yeast, salt, and sugar. After kneading the dough, took the 5 g dough sample from the kneaded dough and rested it in a sealed bowl at 30 ±1 °C for 5 min to allow the relaxation of stresses generated during the sample preparation. The measurements were performed using 35 mm P35 Ti L parallel plates. Placed the sample between the parallel plates and compressed to reach a gap of 1.5 mm. The dough rheological characteristics was measured using a temperature-dependent test, increasing the temperature from 30 °C to 90 °C at 0.058 C/s. The temperature-dependent test was performed at a strain of 0.1% and frequency 1 Hz. Each sample was tested twice.

4.7 Scanning electron microscopy

The microstructure of dough samples was observed by scanning electron microscope (VEGA II LMU VG3720771CZ). Dough samples were prepared by mixing flour and water (90 %). Samples were prepared without enzyme and with enzyme addition format. After lyophilization samples were cut into very small pieces and mounted on metal specimen stubs with double-sided conductive tape. Subsequently, the samples were sputter-coated with gold and observed under an accelerating voltage of 20 kV. Representative pictures were photographed at magnifications of 5000 x. Each sample was tested twice.

4.8 Bread baking Process and analysis

4.8.1 Bread baking

The ingredients' amounts were related on the dry flour basis. Active dry yeast was reactivated for 10 ± 1 min in a saccharose solution at 85% RH and 35 ± 1 ^{0}C temperature. All ingredients were placed into the bowl of the ETA kneader (ETA a.s. Gratus, Czech Republic) and were kneaded for 6 ± 0.5 min. The water addition percentage was the same for all formulas. After kneaded the dough, weighted the dough. After that, the dough was allowed a rise in a proofer at 30 ± 1 0C and a relative humidity of 80 ± 5% for 40 ± 1 min. After that, the loves loading into the baking oven and then baked for 40 ± 2 min at 220 ± 5 ^{0}C in a laboratory baking oven with the proofer (MIWE cube Pekass s.r.o. Plzeň, CZ). The dough and bread-making procedure for all different types of rice flour was the same as the first one [3]

4.8.2 Loaf specific volume

Loves were weighted and loaf specific volume was determined in triplicate by using the rape-seed size plastic granulates.

$$\text{Specific Loaf Volume(mL/g)} = \frac{Loaf\ volume}{Loaf\ weight} \qquad (1)$$

$$\text{Baking Loss (\%)} = \frac{Dough\ weight - bread\ weight}{Dough\ weight} . 100 \qquad (2)$$

4.8.3 Crumb texture analysis

The textural properties of bread crumb were measured using texture profile analysis (TPA) on texture analyzer TA. XT plus (Stable Micro System Ltd.UK). The bread slicer cut each bread sample at 12 mm thickness. Each bread slice cut in 30 mm diameter by using a round stainless-steel cutter. The sample was compressed twice to 4 mm on the base of the analyzer base. The pre-test speed of the test was 5 mm/s. The hardness, springiness, resilience, chewiness, and cohesiveness were calculated.

4.9 Statistical analysis

With the help of analysis of variance (ANOVA), the TPA of the dough and bread results were statistically analyzed. LSD test was used for the differences testing. The differences significance level is tested on $p < 0.05$. The Statistica software was used for the analysis [29].

5. RESULTS

5.1 Dough Rheology

5.1.1 Behavior of dough during uniaxial elongation (Long-deformation)

The impact of α-amylase addition on dough resistance to extension was significant only in doughs prepared from extra-fine and 0.5 S flour. The resistance to extension (R) decreased from 0.26 N to 0.11 N and 0.16 N to 0.09 N upon α-amylase addition in the extra-fine and 0.5 S doughs, respectively. White sticky dough exhibited the highest resistance to extension. The presence of the enzyme decreased the ability of the dough to be elongated. it was not possible to measure the elongation, as shown in Tab 5.1. The extra-fine flour and 0.5 S flour were very fine, as observed by physical touch. The R of 0.5 S dough was lower than that of the extra-fine flour, which may be due to the flour particle size, damaged starch molecules and water absorption capacity of the dough. It may be expected. The extensibility (L) was not significantly different except for the black, and 0.5 S, doughs. The L values of the black (6 mm), and 0.5 S (9 mm), doughs were significantly decreased by enzyme addition (by 3 mm, and 3 mm, respectively). The effect of added enzyme was not significant in the other doughs. The highest R (0.26 N), A (1 N.mm) and L (9 mm) were observed in the extra-fine dough control, as shown in Table 5.1. The impact of α-amylase enzyme addition on dough behavior was weak during the uniaxial deformation test because only mechanically damaged granules may be attacked by α-amylase at laboratory temperature. This impact was observed in finer flours because the content of damaged starch granules were higherin these flours.

Table 5.1 Dough behavior during uniaxial elongation test.

Flour (Rice)	α-Amylase addition (%)	R (N) (Means)	Area (N.mm) (Means)	L (mm) (Means)
Fine flour	0	$0.09{\pm}0.01^{a}$	$0.24{\pm}0.05^{cd}$	$6.00{\pm}0.60^{ef}$
Fine flour	2	$0.07{\pm}0.03^{a}$	$0.13{\pm}0.07^{bc}$	$5.00{\pm}2.00^{ef}$
0.5 S	0	$0.16{\pm}0.21^{b}$	$0.60{\pm}0.07^{g}$	$9.0{\pm}0.40^{g}$
0.5 S	2	$0.09{\pm}0.01^{a}$	$0.11{\pm}0.03^{ab}$	$3.00{\pm}1.00^{bc}$
Semi-coarse	0	N/P	N/P	N/P
Semi-coarse	1.5	N/P	N/P	N/P
Extra fine	0	$0.26{\pm}0.02^{c}$	$1.00{\pm}0.08^{i}$	$9.00{\pm}0.80^{g}$
Extra fine	3	$0.11{\pm}0.06^{a}$	$0.39{\pm}0.03^{f}$	$8.00{\pm}1.00^{g}$
Red	0	$0.08{\pm}0.01^{a}$	$0.08{\pm}0.01^{ab}$	$3.00{\pm}0.50^{bc}$
Red	1	$0.10{\pm}0.01^{a}$	$0.05{\pm}0.01^{ab}$	$1.00{\pm}0.50^{ab}$
Black	0	$0.09{\pm}0.01^{a}$	$0.26{\pm}0.06^{de}$	$6.00{\pm}1.00^{ef}$
Black	2	$0.08{\pm}0.01^{a}$	$0.13{\pm}0.02^{b}$	$3.00{\pm}0.60^{cd}$
Sticky white	0	$0.41{\pm}0.04^{d}$	$0.88{\pm}0.10^{h}$	$5.00{\pm}1.00^{de}$
Sticky white	0.1	N/P	N/P	N/P

R: dough resistance to extension; A: extension area; L: dough extensibility, the mean values followed by different letters in the column differ significantly ($p < 0.05$). N/P- test was not performed.

5.1.2 Dynamic oscillation test of different types of rice flour doughs (small deformation)

This study evaluated the dough behavior on the heat in the different small deformation properties (*G' G"* and *η'*) of all different rice flours (used in study) both (control and enzyme addition) samples.

It has been determined that the behavior of dough on the heat is demonstrated in the different small deformation properties (*G' G"* and *η')* for all different rice flours (used in the study), for both (control and enzyme-added) samples. The heat- dependent variations in the storage modulus (*G'*) and loss modulus (*G"*) for all control dough samples and enzyme-added dough samples respectively. As a resultof the addition of α-amylase enzyme in this study, the red and white-sticky doughs'viscous modulus (*G"*) was increased for the enzyme-added dough sample over the control dough sample. The fine, extra-fine, and black dough samples had a reduced viscous modulus after enzyme addition.

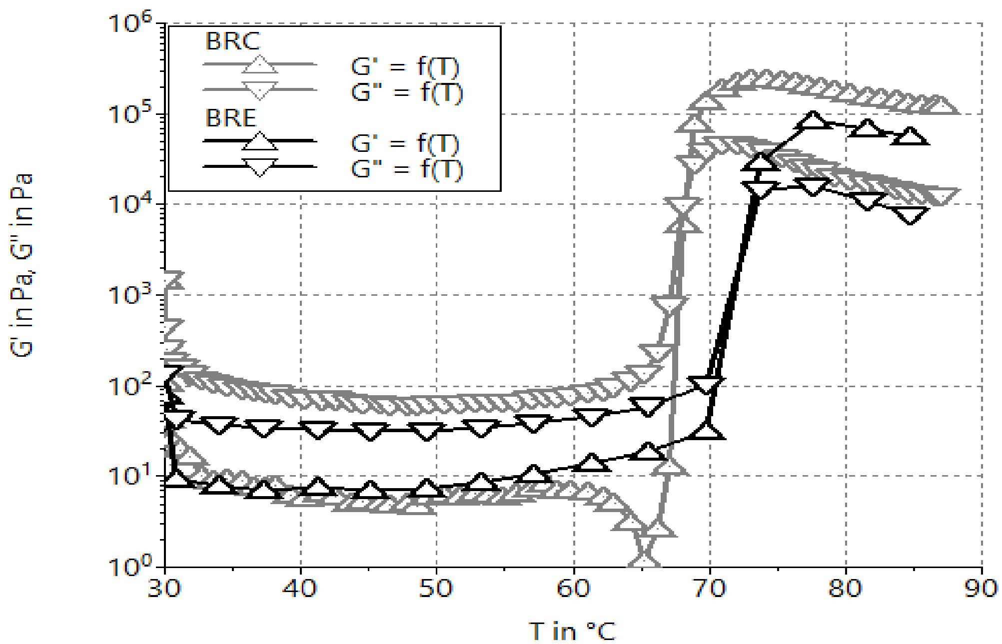

Fig 5.1: Effect of α-amylase addition on G' and G" in black rice doughs samples and comparison with control doughs samples on heat dependent.
(BRC: Black Rice control, BRE: Black rice with enzyme)

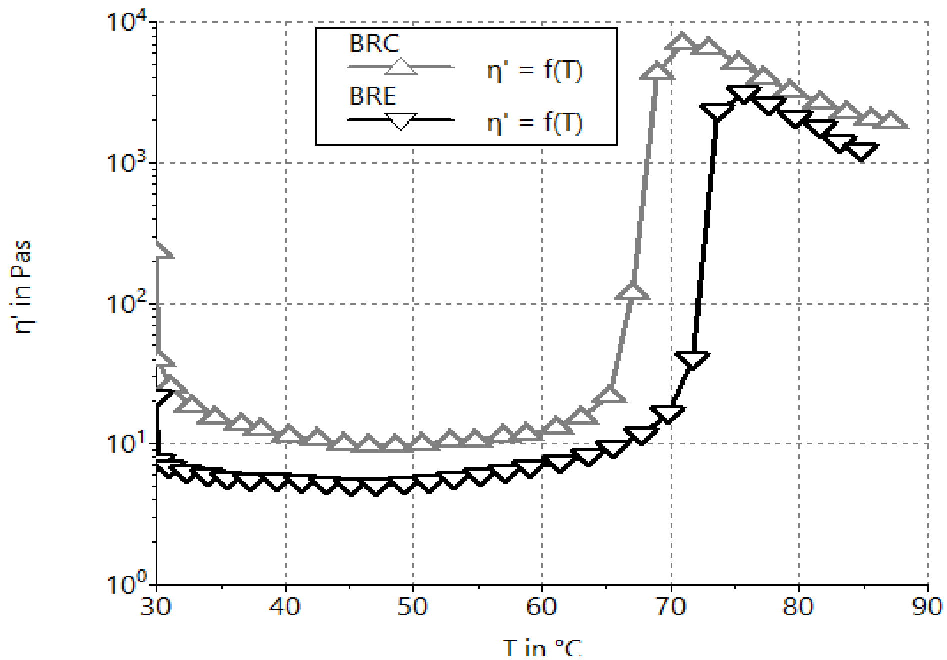

Fig 5.2: Effect of α-amylase addition on η' on black rice dough sample and comparison with control dough sample on heat dependent.

After enzyme addition, elastic modulus (*G'*) was decreased in all samples except for red and white-sticky doughs. A significant increase in the viscosity of the 0.5S, red, and white-sticky dough samples (*η'*) was observed when enzyme was added as compared to the control dough sample. Despite enzyme addition, all the measured samples' elastic modulus was less than their viscous modulus except for the 0.5S dough sample. In the presence of only 0.5S with enzyme, the viscoelastic modulus of dough samples was significantly higher than the viscous modulus (*G''* > *G'*). The oscillation test was not conducted on both semi-coarse dough samples (control and enzyme-treated). It appeared that the flour particles (as seen physically) were larger in size than others in the dough. As a result, the dough's capability of absorbing water was relatively low, and less damaged starch had been included in the flour compared to other flours tested. Prior to heating, the α-amylase enzyme attacks only the damaged starch granules. Except for semi-coarse dough samples, all other tested dough samples showed significant changes in all rheological parameters after enzyme addition in comparison to the control. Using black rice as a control, the elastic modulus (*G'*) was higher than the enzyme-added samples with means of 14000 Pa. Similarly, the viscous modulus (*G''*) of control dough samples was higher than that of enzyme-added samples.

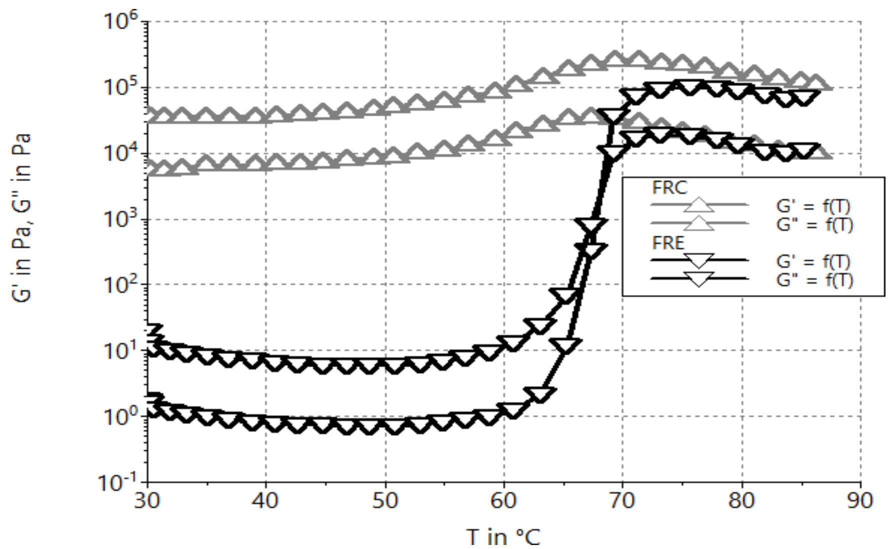

Fig 5.3: Effect of α-amylase addition on G' and G" in fine rice flour doughs samples and comparison with control doughs samples on heat dependent. (FRC: Fine rice control, FRE: Fine rice with enzyme)

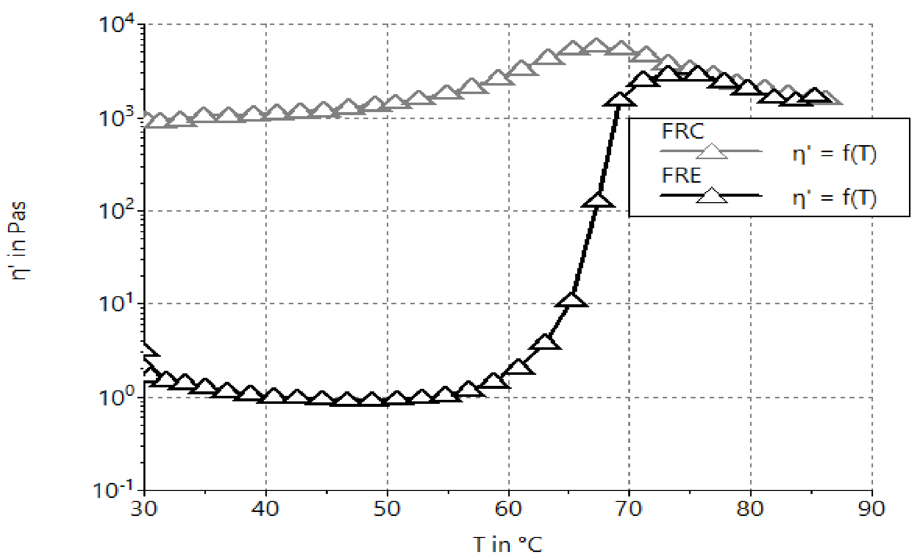

Fig 5.4: Effect of α-amylase addition on η' on fine rice flour dough sample and comparison with control dough sample on heat dependent.

it shows the elastic behavior in both doughs' samples (control and with enzyme addition fine rice dough). Decreases in *G', G"*, and viscosity had showed the soft dough texture. It shows the control dough was less elastic than the enzyme added sample.

5.2 SEM (scanning electron microscopy)

Representative SEM micrographs of control and enzyme added dough as shown in Fig 5.5. The structure of enzyme added samples (extra fine, fine, 0.5S, and red) were significantly different from that of the control samples, the less and complexed starch molecules appeared in the control sample, which resulted in more dense and compact structure. This phenomenon probably decreased the specific volume of the bread made by control rice doughs. After enzyme addition the swollen damaged starch granules were completely different from the control dough samples shown on in Fig 5.5.

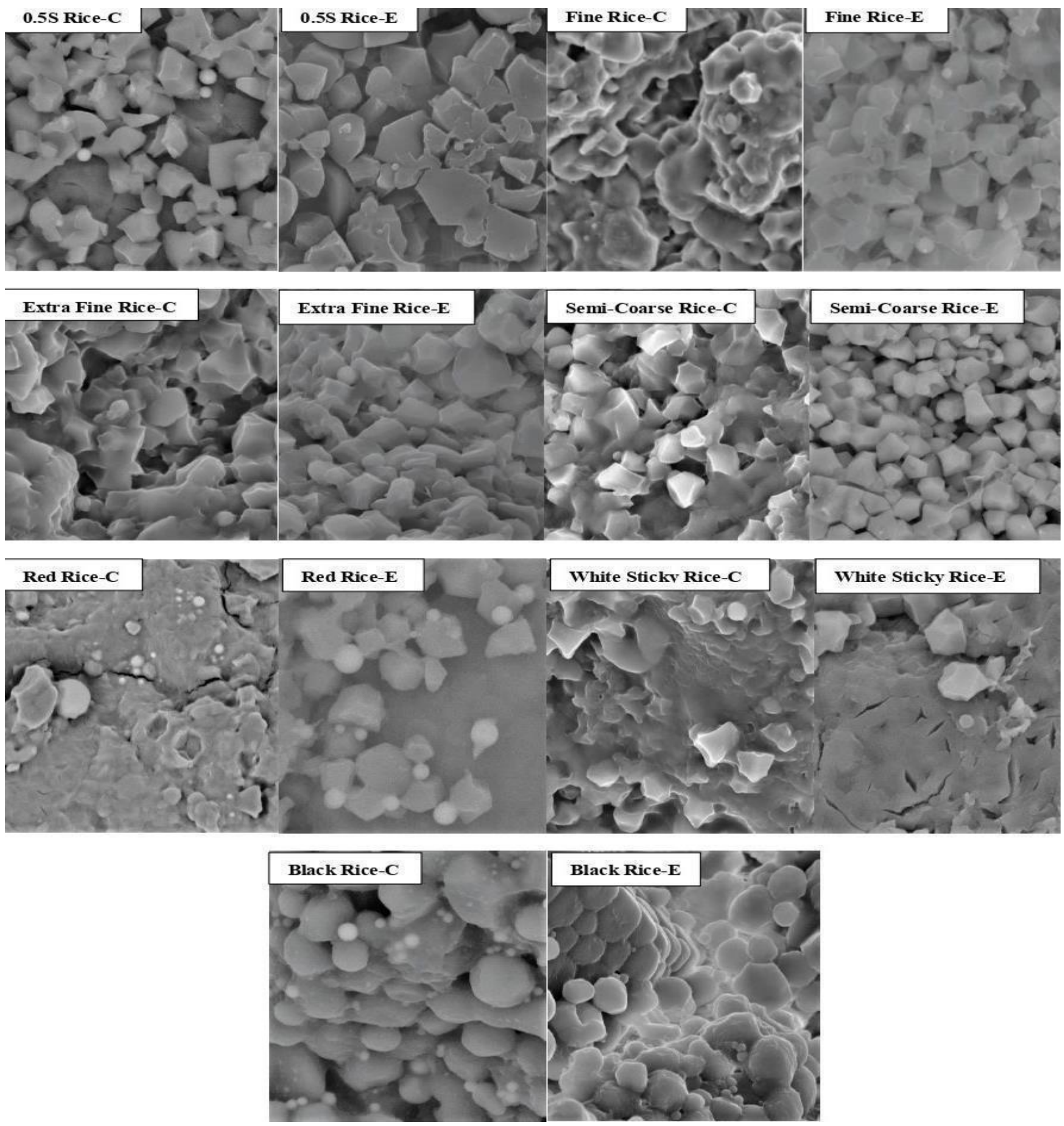

Fig 5.5: Scanning electron micrographs (SEM) of the doughs after kneading (control andwith enzyme addition) at the higher magnification of 5000 X. C- Control, E- With enzyme

6. BREAD EVALUATION

6.1 Texture profile analysis of bread

After enzyme addition, a significant difference in hardness results was observed in the fine flour, semi-coarse, and extra-fine breadcrumbs. The crumb hardness of the control fine flour (127.21 N), semi-coarse (26.69 N), and extra-fine (27.20 N) samples were decreased by α-amylase addition by 2.48, 34.13, 21.35, and 5.46 N, respectively. Except for the fine flour, extra-fine and 0.5 S flour, no other bread had a significant change in springiness. The springiness of the 0.5 S control bread (76.74%) was significantly decreased (62.86%) by enzyme addition. However, the springiness (73.73%) of the extra-fine control bread was increased (98.26%) by enzyme addition. Except for the fine flour and extra-fine breadcrumbs, there was no significant change in other bread crumb samples. The chewiness of the fine flour control bread crumb (613.17) and extra-fine control bread crumb (154.94) was decreased by α-amylase enzyme addition (148.44 and 40.16, respectively) as shown in Table 5.9. The crumb resilience of the extra-fine and semi-coarse breads with enzyme additionwas significantly greater than that of the control bread. The resilience of the controlextra-fine (40.13%) and semi-coarse (38.68%) breadcrumbs was increased byenzyme addition (50.30% and 52.01%, respectively). This result may have occurredbecause the addition of amylase degraded the damaged starch in the dough into smaller dextrins, which were subsequently fermented by the yeast. Last, a significant difference was observed inthe resilience of the fine flour bread crumb. However, the resilience of the enzyme-added fine flour bread crumb (60.15%) was lower than that of the control fine flourbread crumb (73.97%). Gluten-free bread is a complex system, so it is not realisticto generalize that the addition of amylase enzyme to rice dough would produce good-quality bread.

7 DISCUSSIONS

The obtained results from the dough rheology had shown that the 0.5S and extra fine flour dough had a significant difference in resistance to extension and area. Extensibility results had not significant change in extra fine flour dough after enzyme addition, however, after enzyme addition black rice and 0.5S dough had significant changes in extensibility. Only mechanically damaged starch granules be attacked by α-amylase if the dough temperature is below the gelatinization temperature, i.e., during the uniaxial deformation test and other tests performed on dough [30]. This might be related to its ability to catalyze the oxidation of glucose to gluconic acid and hydrogen peroxide, which, in turn, induces the gelation of water-soluble pentosans in rice flour [83]. After enzyme addition in the dough Obtained results from the baking found a significant difference in specific loaf volume only in extra fine flour dough bread. α-Amylase produced an increase in the specific volume [10]. Native starch generates higher solubility than the cross-linked starches formed during baking, giving a shorter texture because it decreases breaking and solubilization, and the gel strength of baked amylose decreases due to the increase in cross-linking [6]. While small deformation results shows that the extra fine flour dough (control and enzyme added) has elastic behavior than other rice flours doughs. After enzyme addition the red rice, and white sticky rice flour dough viscosity was increased, conversely after enzyme addition the black, fine, 0.5 S, extra fine and semi-coarse rice flour doughs viscosity was increased. Therefore, the small deformation results relate to the uniaxial elongation results. As shown in Table 5.1, the extra fine flour dough extensibility has a significant difference after enzyme addition than other dough samples. The R (resistance to extension) values in extra fine flour dough and 0.5 S flour dough have significant differences after enzyme addition. The same changes were observed in baking test (bread loaf specific volume and crumb characteristics) as shown in Table 5.8 and 5.9. The results shows that the impact of α-amylase enzyme was significantly in extra fine flour and 0.5 S flour doughs and bread. Gluten-free bread often has a poor color, mediocre texture, and other problems related to volume and crumb texture. The absence of gluten often results in excessively fluid doughs, which are closer in viscosity to cake batters, with a lower gas holding capacity than wheat flour doughs. Choosing the correct type ofrice flour is essential, since different flours can have different impacts on the final quality of products [31], [32][33].

7. CONTRIBUTION TO SCIENCE AND PRACTICE

The study effect of enzyme on the rheological properties of Gluten-free flour and bread is very perspective area. The research on this field is promising new approaches how to understand the effect of enzyme on functional character of gluten-free dough and bread and how to optimize their amount in selected food matrices.

The main contributions of this Doctoral Thesis in the field of effect of enzyme on gluten free dough and bread rheology can be summarized into the following points:

- Conformational ordering of alpha amylase enzyme addition induced by temperature changes influences their rheological behavior and other functional properties of the dough.

- In Dynamic oscillation test, after enzyme addition $G'<G''$ was observed in 0.5S and fine rice dough. It means the dough was more viscous than the control doughs. The action of α-amylase on damaged starch results in a release of absorbed water, resulting in a lower storage modulus

- Addition of alpha amylase enzyme in the dough used affects the storage modulus, loss modulus and viscosity behavior of the dough.

- After enzyme addition the dough texture and extensibility were significantly changed in all gluten free dough.

- The ability of flour samples to bind moisture in varying degrees depends on the particle size of the flour and higher-order structure of each flour studied.

- In dependence of the alpha amylase enzyme activity approach used (Hagberg falling number meter), the values of alpha amylase enzyme (*FN*) vary in a specific range. After alpha amylase enzyme the activity of the enzyme was increased.

- There was found a significant change between control dough sample and after enzyme addition rheological characteristics of the glute free rice flour dough. The highest significant changes in the rheological characteristics after enzyme addition was observed in extra fine flour, fine flour and 0.5S flour dough samples.

8 CONCLUSION

The objective of the dissertation thesis was to evaluate the impact of the α-enzyme on the rheological properties and texture of final products manufactured by the different types of rice flours dough (fine, extra-fine, semi-coarse, 0.5S, red, black, white-sticky) samples. The absence of gluten, whose presence determines the overall appearance and textural properties of bread making, makes it a technological challenge. The role of enzyme is improving the functionality of gluten-free doughs, bread, and bakery products. The enzyme addition increases the textural and rheological properties of some rice flour doughs. Obtained results support that the enzyme addition could increase the quality of the final product (bread) from the extra fine, and fine flour dough breads. The effects of a-amylases on dough and bread rheological properties were not the same. Dough Texture profile analysis results shows that the extra fine, 0.5S and black rice flour dough had a significant change. After enzyme addition extra fine flour dough had not change in extensibility, conversely, 0.5S and black rice doughs had significant change in extensibility. Except 0.5S, black, and extra fine flour doughs no other tested flour dough had significant changes in texture profile analysis. In oscillation test of the dough after enzyme addition extra fine, fine and black rice flour doughs storage, loss modulus and viscosity were decreased, conversely, after enzyme addition 0.5S flour dough storage modulus was increased. Except after enzyme addition 0.5S flour dough, all both (control and with enzyme) dough samples storage modulus were greater than loss modulus. α-amylase added dough samplesstarch granules were small and visible in microstructure to compare than control dough samples starch granules. It shows the enzyme react on the damaged starch granules and does hydrolyze the starch molecules. In microstructure results the control dough samples; the starch granule microstructures were complex and noteasily visible. The obtained baking results (loaf specific volume and the TPA results) of final product were significantly different from the control doughs sample. It can be assumed that the α -amylase enzyme can be utilized to develop fine rice flour products. It is possible to improve the quality of rice flour dough breads by adding alpha-amylase enzyme.

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LIST OF SYMBOLS AND ABBERVATIONS

RF	Rice flour
GF	Gluten-free
GFB	Gluten-free bread
α	Alpha
C	Control (without enzyme)
E	with enzyme
G’	Elastic Modulus
G”	Viscous Modulus
ɳ’	Viscosity
BRC	Black rice dough control
BRE	Black rice dough with enzyme
FRC	Fine rice dough control
FRE	Fine rice dough with enzym2

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The Effect of Alpha-amylase Enzyme Addition on the rheological Characteristics of the Gluten-free Dough and Bread

Vliv přídavku enzymu na reologické vlastnosti bezlepkového těsta a pečiva

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