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Effect of Processing Variables and Enzymatic Activity on Wheat Flour Dough Extruded Under Different Operating Conditions

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Summary

Low processing temperatures are required to improve the texture of products when enzymes are directly added to the extruder. Interaction among processing variables and enzymatic activity can occur during extrusion. In this research, the influence of some extrusion parameters (barrel temperature, dough moisture and screw speed) on the activity of two commercial enzymes (Grindamyl Amylase 1000 and Grindamyl Protease 41) has been studied. Wheat flour was used as a model system, and macromolecular degradation was determined by water solubility index (WSI). Moreover, gelatinization degree and die pressure were evaluated. Results showed that barrel temperature affected enzyme activity. High values of WSI were obtained at high barrel temperature using Grindamyl Protease 41. When Grindamyl Amylase 1000 was used, low values of starch gelatinization were obtained. The activity of both enzymes was negatively affected by high values of dough moisture.

Key words: amylase, enzymes, extrusion, protease, wheat flour

Introduction

The use of enzymes in baking technology is a very common practice (*1,2*). They are responsible for the improvement of dough machinability and bakery product quality. In particular, α-amylases favour the production of fermentable sugars that increase fermentation rate, oxidases and proteases increase dough stability, proteases favour the reduction of dough mixing time and enhance dough extensibility, while oxidases reduce it (*3*).

The optimization of dough rheological characteristics improves the quality parameters of bakery products. Significant improvements were obtained by adding flour with enzymes: an increase in bread volume, improvement in crispness and colour of the crust, softness of crumb grain and overall better sensory characteristics were achieved (*4*). Moreover, the enzymes slow down the crumb firming process and increase the shelf-life of products (*5*).

The possibility of using an extruder as a continuous bioreactor in which materials can be treated in the presence of enzymes under elevated temperature, pressure and shear stress at various moisture levels in cereal grain and flour has been studied (*6*). Most researchers studied the possibility of replacing the conventional method of glucose syrup production (which involves the acidic hydrolysis of starch) with an enzymatic treatment of gelatinized starch by extrusion-cooking process. This treatment requires the direct addition of heat stable α-amylases (extracted from *Bacillus licheniformis*) during extrusion

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(*7,8*). Furthermore, the presence of thermolabile ingredients or fat flour requires low processing temperatures with a consequent lack of product expansion: this could be avoided using some enzymes that could improve product texture.

De Pilli *et al.* (*9*) studied the effects of two commercially available enzymes (protease and amylase) on the texture of products obtained from wheat and almond flour extruded at low temperature (54 °C). Results showed that protease affected the rheological characteristics of dough (rapid drop of consistency during farinographic analyses) and improved the structure of extrudates with high lipid content (lower break strength, high porosity and water solubility index). Nevertheless, the effect of process variables on enzymatic activity was not investigated.

Having this in mind, the aim of this work is to investigate the effect of some extrusion-cooking parameters (barrel temperature, dough moisture and screw speed) on the activity of two commercial enzymes (Grindamyl Amylase 1000 and Grindamyl Protease 41). The effects of interaction between processing variables and enzymatic activity on the die pressure (processing parameter), macromolecular degradation (water solubility index and water absorption index) and gelatinization degree of wheat flour (used as model system) were investigated. Moreover, the effect of enzyme dosage was considered since it had a significant effect on the process costs.

Wheat flour was used as a model system since most extruded products, such as snacks or breakfast cereals, are made with this type of flour (*10*). Water solubility index (WSI) was chosen because of its suitability to evaluate the macromolecular degradation of biopolymers that occurs during extrusion process (*11,12*). Water absorption index (WAI) is related to the volume of starch grain (swollen because of hydration), which preserves their wholeness in a water solution (*13*).

Materials and Methods

Flour

Wheat flour was supplied by Cereal Destrine (Cadelbosco Sopra, Reggio Emilia, Italy) and its chemical composition is reported in Table 1.

Enzymes

Grindamyl Amylase 1000 from *Aspergillus oryzae* (1000 fungal amylase units (FAU) per g) and Grindamyl Protease 41 from *Bacillus subtilis* (41 FAU/g) were used, both supplied by Danisco Cultor (Grindsted, Denmark).

Measurement of enzyme activity

Grindamyl Amylase 1000 (GA amylase) activity was assayed as described by Rani *et al.* (*14*). A volume of 1 mL of substrate (1 g of soluble starch in 100 mL of distilled water) was incubated with 1 mL of different concentrations of the enzyme (0.025, 0.05, 0.075 and 0.1 g in 10 mL of 0.016 M sodium acetate buffer at pH=4.8) for 3 min. The enzyme reaction was stopped by the addition of 2 mL of dinitrosalicylic acid reagent. The tubes were heated for 5 min in a boiling water bath until colour was developed and then they were cooled. The solution was made to the required volume and the absorbance was measured at 540 nm (by a Shimadzu UV-1201 UV/VIS spectrophotometer, USA) using maltose as standard. The results were expressed as mg of maltose liberated in 3 min at 37 °C by 1 mL of enzyme solution.

Grindamyl Protease 41 (GA protease) assay was carried out as described by Rani *et al.* (*14*). Azocasein substrate (25 mg) was dissolved in 1 mL of 50 mM sodium phosphate buffer (pH=7.5). A volume of 450 μL of sodium phosphate buffer (50 mM, pH=7.5) was added to 50 μL of substrate solution and preincubated for 10 min at 37 °C. A volume of 1 mL of enzyme solutions (0.25, 0.5, 0.75 and 1 g in 10 mL of sodium phosphate buffer) was added and the mixture was incubated for 30 min at 37 °C. The reaction was terminated by adding 0.5 mL of 10 % trichloracetic acid (TCA) and the precipitate was removed by centrifugation at 8000×*g* for 10 min at 4 °C. A volume of 40 μL of NaOH (10 mM) was added to the supernatant and the absorbance was read at 440 nm using a Shimadzu UV-1201 UV/VIS spectrophotometer, USA. One unit of activity was defined as the change of 1 unit in absorbance.

Chemical analysis of the flour

The content of moisture, ash, protein, fat, *etc.* were determined according to AACC methods, sections 44-15A (*15*).

Experimental design

The factorial design of five variables (barrel temperature, screw speed, dough moisture expressed as percentage of dry mass, mass fractions of GA amylase and GA protease) and five levels obtained by a central composite design (CCD) (*16*) are shown in Table 2. This factorial design, which allows reducing the number of possible combinations to a manageable size, was used to evaluate the individual influence of the processing variables and their possible interactions. The activity of GA amylase and GA protease for each mass fraction used in this work is reported in Table 3. Thirty tests with different combinations of process variable values (barrel temperature, moisture dough, screw speed, mass fractions of GA amylase and GA protease) were obtained using the following equation:

$$N_{tot}=N_0+N^*+N_c=16+10+4=30 \qquad /1/$$

where $N_0=2^{N-1}$, N is the number of variables, N_c is the number of central points and N^* is the number of star points (*16*). The obtained combinations are reported in Table 4.

The appropriate range of processing parameters and amounts of enzymes were chosen according to De Pilli *et al.* (*9*).

Extrudate formula

The dough was prepared using wheat flour, tap water and different mass fraction of enzymes as reported in Table 2.

Table 1. Chemical composition of flour used for the extrusion experiments

Raw material	*w*(moisture)/%	*w*(ash)/%	*w*(protein)/%	*w*(lipid)/%	*w*(carbohydrate)/%	*w*(starch)/%	*w*(dried gluten)/%
Wheat flour	11.61	0.518	9.57	1.0	76.2	67.7	5.46
S.D.	0.04	0.01	0.07	0.103	0.201	0.110	0.2

S.D.=standard deviation

Table 2. Central composite design

Codified value	*t*(extrusion)/°C	*w*(DM)/%	v_s/rpm	*w*(amylase)/(g/kg)	*w*(protease)/(g/kg)
–2	60	20	100	0	0
–1	75	24	175	0.025	0.25
0	90	28	250	0.05	0.5
1	105	32	325	0.075	0.75
2	120	36	400	0.1	1.0

Table 3. Enzyme activity

w(GA amylase)/(g/kg)	Enzyme activity/(U/g)	*w*(GA protease)/(g/kg)	Enzyme activity/(U/g)
0	0	0	0
0.025	2.09	0.25	0.271
0.05	2.79	0.5	0.357
0.075	3.57	0.75	0.468
0.1	4.27	1.0	0.49

Table 4. Experimental factorial design

Sample	*t*(extrusion)/°C	*w*(DM)/%	v_s/rpm	*w*(amylase)/(g/kg)	*w*(protease)/(g/kg)
1	75	24	175	0.5	1.5
2	75	24	175	1.5	0.5
3	75	24	325	0.5	0.5
4	75	24	325	1.5	1.5
5	75	32	175	0.5	0.5
6	75	32	175	1.5	1.5
7	75	32	325	0.5	1.5
8	75	32	325	1.5	0.5
9	105	24	175	0.5	0.5
10	105	24	175	1.5	1.5
11	105	24	325	0.5	1.5
12	105	24	325	1.5	0.5
13	105	32	175	0.5	1.5
14	105	32	175	1.5	0.5
15	105	32	325	0.5	0.5
16	105	32	325	1.5	1.5
17	60	28	250	1.0	1.0
18	120	28	250	1.0	1.0
19	90	20	250	1.0	1.0
20	90	36	250	1.0	1.0
21	90	28	100	1.0	1.0
22	90	28	400	1.0	1.0
23	90	28	250	0	1.0
24	90	28	250	2.0	1.0
25	90	28	250	1.0	0
26	90	28	250	1.0	2.0
27	90	28	250	1.0	1.0
28	90	28	250	1.0	1.0
29	90	28	250	1.0	1.0
30	90	28	250	1.0	1.0

Extruder characteristics

A BC-21 Clextral (Firminy, France) co-rotating twin-screw extruder was used. The screw geometrical features were the following: diameter 25 mm and length 900 mm, distance between shafts 21 mm (L/D=36:1). The used screw configuration (from inlet to the die) is reported in Table 5.

During extrusion experiments, the flour feed rate was maintained constant at 10 kg/h using a volumetric gravity feeder, while the moisture content of dough was adjusted using a water pump that pumped the water into the first zone of extruder. The extruder was divided into nine zones independent of each other for temperature control and adjustment. The first five zones were kept at room temperature, whereas the last four zones were adjusted to the temperatures reported in experimental factorial design (Table 4).

Table 5. Screw profile used for extrusion experiments

Type of screw element	Screw element details (pitch/length)	Total length
		mm
Forward pitch	50/50	150
	50/33	100
Kneading block	50/25	200
	50/33	100
	50/25	100
Decreased pitch	50/16	250

The die pressure during extrusion was measured with a pressure transducer (Dynisco PT462, Milan, Italy). A spherical die (diameter 60 mm) provided with two holes (spherical shape and diameter of 5 mm each) was used.

Extrudates were cut into pieces through a cutter (set at 210 rpm) at the exit of the die, put on aluminium trays and dried at 80 °C for 10 min in an oven (Delonghi Combi 8 Functions Convection, Milano, Italy). After drying, extrudates with 8 % moisture content were stored in polyethylene bags and analysed.

Determination of water solubility index (WSI) and water absorption index (WAI)

Water solubility index of extrudates was determined following the method of Anderson *et al.* (*17*). The extrudates were ground and sifted (180 and 250 microns). Then, 30 mL of distilled water were mixed with 2.5 g of powder for 30 min by a magnetic agitator. A mass of 32.5 g of this suspension was centrifuged at 3000 rpm for 10 min (ALC model 423R, Milan, Italy). The liquid phase was placed into an aluminium capsule and dried at 105 °C until constant mass. The WSI was determined from the dry extract using the following formula:

$$\mathrm{WSI} = \frac{m(\text{dry extract})}{m(\text{extrudate})} \cdot 100 \qquad /2/$$

The remaining gel was weighed and the WAI was calculated as follows:

$$\mathrm{WAI} = \frac{m_g}{m_{ds}} \qquad /3/$$

where m_g is the mass of gel and m_{ds} is the mass of dry sample.

Degree of starch gelatinization

Starch gelatinization degree was determined at 600 nm through a DU 640 spectrophotometer (Beckman Instruments, Fullerton, CA, USA) following the method described by Wootton *et al.* (*18*).

Statistical analysis

All data were submitted to statistical analysis by Winstat v. 6.0 software (Statsoft, Tulsa, USA). The mathematical modelling was carried out in two steps. The first step involved a stepwise regression to identify the relevant variables; the second step consisted of a multiple regression (standard least square fitting) to fit a second order mathematical model, according to the following polynomial equation:

$$y = B_0 + \Sigma B_i \chi_i + \Sigma B_{ii} \chi_{ii}^2 + \Sigma B_{ij} \chi i_{ij} \qquad /4/$$

where y is the dependent variable (die pressure, WSI, WAI and starch gelatinization degree), B_0 is a constant value, χ_i and χ_j are the independent variables in coded values, and B_i, B_{ii} and B_{ij} are the regression coefficients of the model. Variables having a significance lower than 95 % ($p>0.05$) were left out of the equation. This model assessed the effects of the linear (χ_i), quadratic (χ_i^2) and combined ($\chi_i\chi_j$) terms of the independent variables (barrel temperature, dough moisture, screw speed, mass fractions of GA amylase and GA protease) on the dependent variable.

In order to describe the individual and interactive effects of the independent variables on the modification of wheat flour macromolecules (in terms of WSI, WAI and starch gelatinization degree), iso-response surfaces were developed, allowing three independent variable constants at the central level of central composite design.

Results and Discussion

Fig. 1 shows the standardized effects of significant independent variables on the die pressure. This parameter was negatively influenced by barrel temperature and dough moisture, *i.e.* pressure decreased at high barrel temperature and dough moisture. Viscosity of molten polymers decreased as barrel temperature increased, resulting in the reduction of die pressure. Since the die pressure is related to melt viscosity at the end of the extruder, it has been used in food industry as a means of extruder control (*19*,*20*). Die pressure decreased when water content increased because of the reduction of molten starch viscosity inside the extruder (*21*,*22*). Water content decrease at the lowest temperature led to a corresponding increase in die pressure. Also, amylase had a negative effect on this parameter (Fig. 1) because of high production of dextrin that reduced viscosity (*23*), and consequently die pressure. The interaction between dough moisture and the mass fractions of amylase showed a positive effect on the pressure, which increased with the increase of dough moisture and mass fraction of amy-

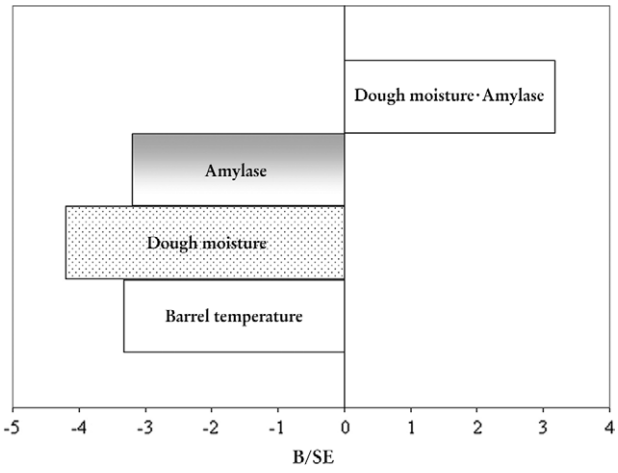

Fig. 1. Standardized effects of significant independent variables on the die pressure

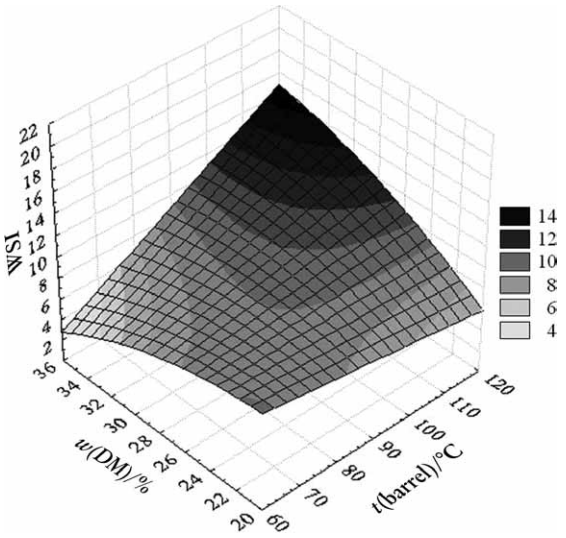

Fig. 2. Water solubility index (WSI) as a function of barrel temperature and dough moisture (DM)
$WSI=24.248-0.3722 \cdot t(\text{barrel})-0.169 \cdot w(\text{DM})-0.0211 \cdot w^2(\text{DM})+0.0159 \cdot (t(\text{barrel}) \cdot w(\text{DM}))$

lase. This trend could be attributed to the decrease of enzymatic activity caused by high moisture content. The heat stability of enzymes increased markedly at low water activity due to the reduction of protein molecule mobility, which inhibited conformational changes, leading to activity loss (*24*). Water is required to maintain the catalytically active conformation of enzymatic system. On the other hand, during the denaturation process, it acted as a plasticizer, which allowed the enzyme molecules to unfold, resulting in the loss of native conformation. Therefore, dehydration made proteins less sensitive to denaturation. Although dried proteins were more rigid, water molecules needed to be replaced since they were essential for maintaining their tertiary structure (*25*).

Fig. 2 shows the WSI values as a function of barrel temperature and dough moisture. A positive effect of these two independent variables on WSI can be observed from the partial equation and iso-response surface, *i.e.* the highest values of WSI were obtained at the highest barrel temperature and dough moisture. This can probably be attributed to a higher gelatinization degree that occurred under these operating conditions, since WSI was directly correlated with gelatinization degree (*11*).

Apparently, these results are in disagreement with those obtained by Singh and Smith (*26*), who observed an increase in WSI with the increase of barrel temperature and a decrease of WSI with the increase in moisture content. Similar effects of decreasing moisture on WSI had been reported earlier for starch, maize grits, wheat and pea flour (*11*,*27*). Mercier and Feillet (*28*) and Kim (*29*) observed a decrease in soluble carbohydrates with the increase in wheat flour moisture. The highest WSI obtained at the highest barrel temperature could be attributed to starch degradation and consequently to the increase of water-soluble carbohydrates (*28*,*30*,*31*). Nevertheless, these researchers carried out their extrusion experiments without enzymes. Tomás *et al.* (*32*) studied the effect of operating conditions on dextrose equivalent (DE) values obtained in enzymatic extrusion: rice starch was extruded in a twin-screw extruder adding a thermostable α-amylase, and then feed moisture content, processing temperature and enzyme concentration were evaluated. Results showed that water content had a relevant effect on DE production; in particular, the highest water content determined the maximum value of DE. Similiar results were reported by Linko *et al.* (*33*), Hakulin *et al.* (*34*), Lee and Kim (*35*) and Likimani *et al.* (*36*), who proved that moisture excess in the feed resulted in complete starch gelatinization if combined with elevated temperatures; therefore, the increase of moisture usually results in a higher degree of starch hydrolysis.

In Fig. 3, the WSI values are reported as a function of the mass fractions of GA amylase and dough moisture. From the related partial equation, a clear positive effect on WSI emerged. In particular, the mass fraction of GA amylase showed a greater effect than dough moisture (high value of angular coefficient) because of high hydrolytic activity of this enzyme. Moreover, a negative interaction between dough moisture and the mass fraction of GA amylase can be observed: a slight decrease of WSI values occurred with the increase of dough moisture and the amount of enzymes. These results are in agreement with Tomás *et al.* (*32*), who concluded that enzymatic hydrolysis in the extrusion processing strongly depended on water content, and in particular, higher water content caused a decrease of enzymatic activity since, as reported above, the increase of moisture feed made the enzyme more sensible to denaturation.

Fig. 4 shows that the highest values of WSI were obtained at high mass fractions of GA protease and barrel temperatures. The observed interaction between GA protease activity and barrel temperature is due to reliance of enzymatic reactions (like chemical reactions) on temperature, as described by the well-known Arrhenius equation. In fact, the speed of enzymatic reaction increases with the increase of temperature until the enzyme preserves its three-dimensional structure and its functionality (*37*).

GA protease activity weakly decreases at the highest dough moisture. The trend of this iso-response surface is supported by the presence, in the partial equation, of a

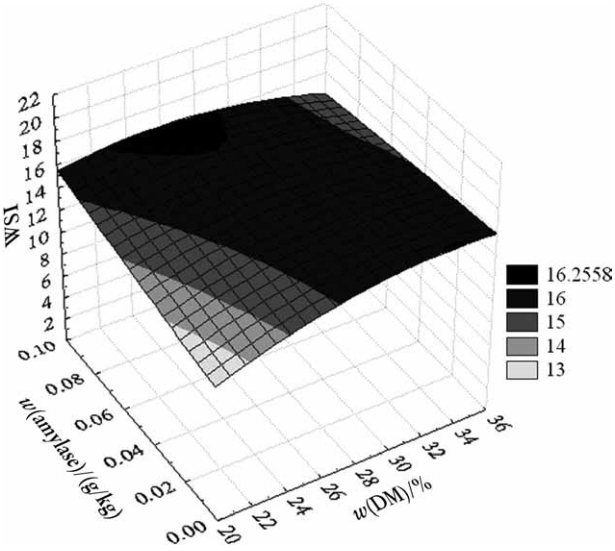

Fig. 3. Water solubility index (WSI) as a function of the mass fraction of GA amylase and dough moisture
WSI=–8.2318–0.0211·w^2(DM)+1.431·w(DM)+103.4464·w(amylase)–
–3.3845·(w(amylase)·w(DM))

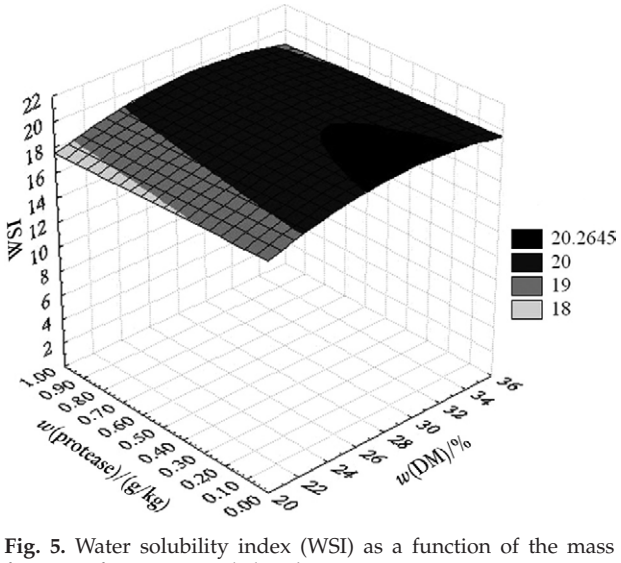

Fig. 5. Water solubility index (WSI) as a function of the mass fraction of protease and dough moisture
WSI=1.3952+1.262·w(DM)–0.0211·w^2(DM)–0.664·w(protease)

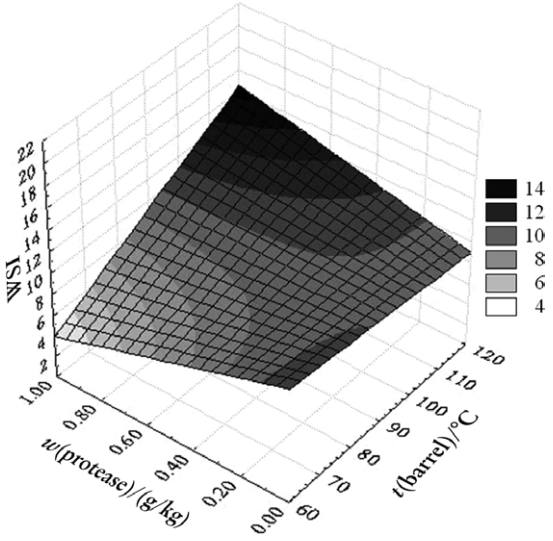

Fig. 4. Water solubility index (WSI) as a function of the mass fraction of protease and barrel temperature
WSI=12.59–0.030·t(barrel)–19.2534·w(protease)+0.2066·(t(barrel)·
·w(protease))

quadratic factor of dough moisture variable that showed no linear correlation with a negative angular coefficient (Fig. 5).

The general equation related to water absorption index (WAI), which was obtained by applying the stepwise backward regression and multiple regression to the experimental data, is the following:

$$\begin{aligned}\text{WAI}=&785.1-11369.3\cdot w(\text{amylase})+\\&+0.2\cdot t^2(\text{barrel})+0.9\cdot w^2(\text{DM})-0.9\cdot t(\text{barrel})\cdot\\&\cdot w(\text{DM})+388.1\cdot(w(\text{DM})\cdot w(\text{amylase}))\end{aligned}\qquad /5/$$

where correlation coefficient is R=0.82 at p<0.001 and w(DM) is dough moisture percentage.

The equation shows that barrel temperature and dough moisture had a significant effect on WAI values. In particular, a negative effect of these operating variables on this index was observed (Eq. 5). This means that the increase of barrel temperature at low dough moisture involves a decrease of WAI values, probably due to an increase in starch degradation. The present observations corroborate those reported earlier (*38*,*39*). Furthermore, WAI generally increases in parallel with the increase of extrusion temperature, probably due to increased dextrinization (*28*,*40*). As expected, the only enzyme activity that had a significant effect was amylase (Eq. 5). In particular, the increase of amylase mass fraction caused a decrease of WAI because of starch degradation in dextrins, which have a scanty ability to bind water in comparison with starch grains.

Fig. 6 shows the degree of starch gelatinization as a function of barrel temperature and dough moisture. From iso-response surface and its partial equation, a positive interaction between independent variables can be observed. As expected, the highest degree of starch gelatinization was obtained at the highest barrel temperature and dough moisture (*41*). This confirms the previous hypothesis related to WSI, *i.e.* the highest values of this index, which were observed at the highest barrel temperature and dough moisture, were determined by high degree of starch gelatinization (Fig. 2).

The increase of screw speed caused a reduction of starch gelatinization degree (Fig. 7) because of considerable dextrinization that occurred under these operating conditions (*8*,*31*,*42*).

The general equation related to starch gelatinization degree, which was obtained by applying the stepwise backward regression and multiple regression to the experimental data, is the following:

$$\begin{aligned}\text{Starch gelatinization degree}=&22.53-1.34\cdot w(\text{DM})+\\&+4229.17\cdot w^2(\text{amylase})+0.02\cdot(t(\text{barrel})\cdot w(\text{DM}))-\\&-5.86\cdot(t(\text{barrel})\cdot w(\text{amylase}))-0.09\cdot(v_s\cdot w(\text{protease}))\end{aligned}\qquad /6/$$

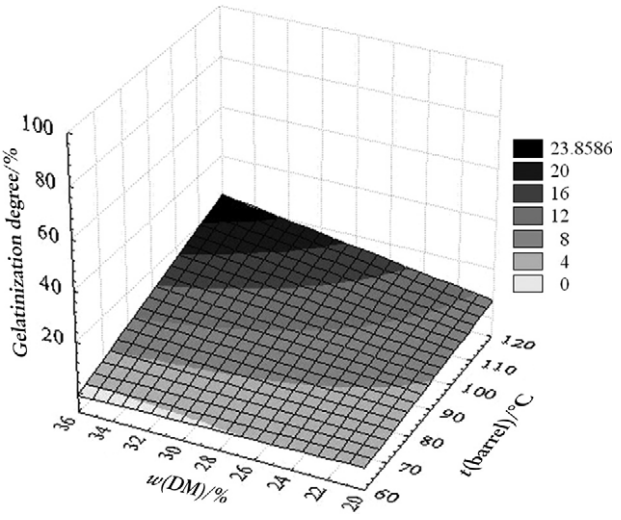

Fig. 6. Starch gelatinization degree as a function of barrel temperature and dough moisture
Gelatinization degree=21.855–1.3444·w(DM)+0.020·(t(barrel)·
·w(DM))–0.3·t(barrel)

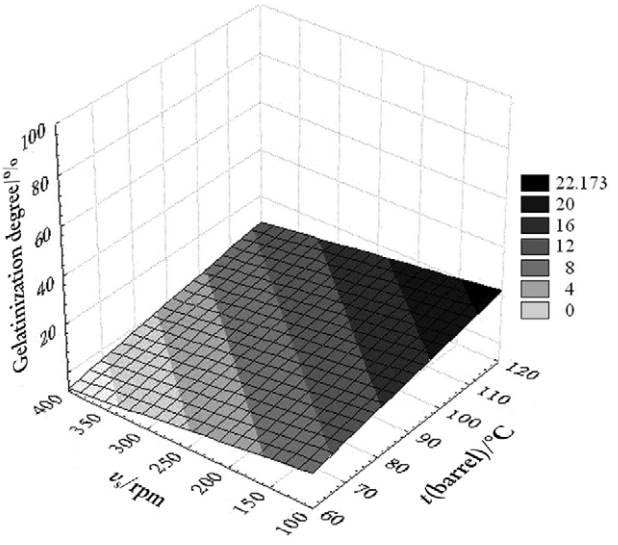

Fig. 7. Starch gelatinization degree as a function of barrel temperature and screw speed
Gelatinization degree=–4.527+0.26·t(barrel)–0.045·v_s

where correlation coefficient is R=0.86 at p<0.001, w(DM) is dough moisture percentage and v_s is screw speed.

In this equation, a negative effect of the interaction between barrel temperature and mass fraction of GA amylase on starch gelatinization degree can be observed. In fact, the increase of barrel temperature favoured the hydrolysis of GA amylase, causing higher dextrinization of starch and reduction of its gelatinization. This effect is shown in Fig. 8, where the starch gelatinization degree quickly decreases with the increase of the mass fraction of enzyme. Moreover, no interaction between enzymatic activity, screw speed (Fig. 8) and dough moisture was observed (data not shown).

Concerning GA protease effect on starch gelatinization degree, a progressive decrease of gelatinization with the increase of enzyme mass fraction was found (Fig. 9).

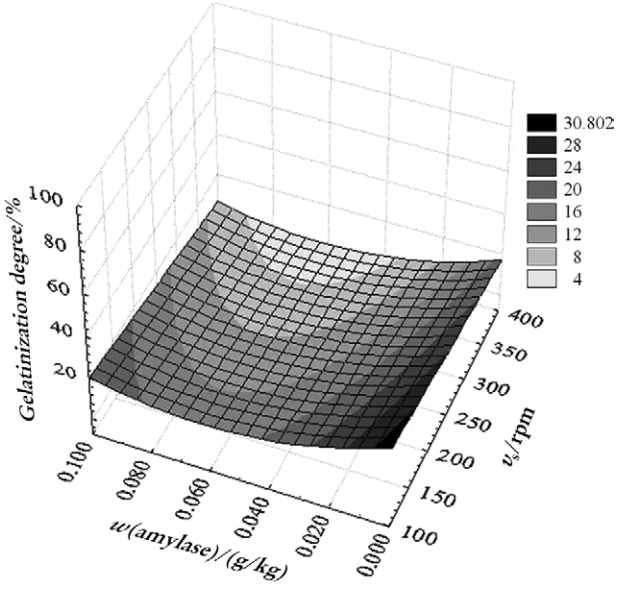

Fig. 8. Starch gelatinization degree as a function of the mass fraction of GA amylase and screw speed
Gelatinization degree=35.302+4229.171·w^2(amylase)–527.31·
·w(amylase)–0.045·v_s

This could be due to the production of hydrosoluble compounds with low molecular mass, which come from partial degradation of gluten caused by GA protease and which competed with starch for water absorption (*9*). Moreover, in Eq. 1 a negative effect of GA protease and screw speed on starch gelatinization can be observed. The increase of screw speed, in fact, caused a mechanical degradation of starch in dextrin because of shear stress and determined a reduction of starch gelatinization. The increase of GA protease activity caused an increase of hydrosoluble compounds with low molecular mass, which competed with starch for water absorption and determined a reduction of starch gelatinization.

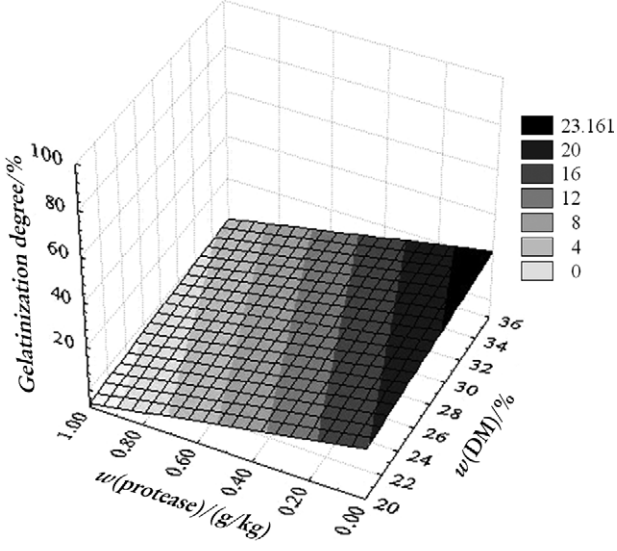

Fig. 9. Starch gelatinization degree as a function of the mass fraction of GA protease and dough moisture
Gelatinization degree=6.745–22.5·w(protease)+0.456·w(DM)

Conclusions

The addition of GA amylase significantly modified the rheological characteristics of dough, above all its viscosity because of macromolecular degradation of starch. On the contrary, GA protease did not have any effect on die pressure, which is related to dough viscosity. GA amylase was mainly influenced by dough moisture, while the barrel temperature lowered its activity due to its thermolability. Instead, the GA protease activity (heat-proof enzyme) was improved by high barrel temperature. Therefore, it is suitable to take these elements into consideration when high processing temperature is applied in the extrusion carried out in the presence of enzymes.

Finally, starch gelatinization was blocked at the highest mass fraction of both enzymes. Since starch gelatinization is essential for the expansion of extrudates, it is advisable to choose the amount of enzymes able to assure an optimal macromolecular modification of dough in order to improve its rheological characteristics without compromising the extrudate structure.

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