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Thermal transitions of gluten-free doughs as affected by water, egg white and hydroxypropylmethylcellulose

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Abstract

The thermal performance of a gluten-free bread dough consisting of a blend of non allergenic corn and cassava starches (75:25) with hydroxypropylmethylcellulose (HPMC) as gluten mimetic hydrocolloid in conjunction with egg white (EW) was determined by differential scanning calorimetry. In order to analyse the effects of different levels of the components (water: 80–110%, HPMC: 0–2% and EW: 0–10% over the starch blend) on the thermal transitions of the dough, a Doehlert design and a response surface methodology were used.

The analysis of variance showed that EW did not affect the onset temperature of gelatinisation and HPMC did not affect the peak and conclusion temperatures. HPMC–water interactions mainly controlled the onset temperature of starch gelatinisation. On the other hand, the peak and conclusion temperatures were determined by the additive and opposite effects of water and EW.

Keywords: Differential scanning calorimetry; Starch; Interactions; Hydrocolloids; Gelatinisation

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1. Introduction

Some individuals need a gluten-restrictive diet in order to avoid the effects of an entheropathy (celiac disease) caused by the intolerance to gliadins normally present in baked foods as bread [1,2]. Wheat starch has been utilised as a replacement for wheat flour; however, many individuals sensitive to the gliadin fraction of gluten proteins cannot tolerate even the very small amount of this protein in wheat starch [3]. Therefore, gluten-free starches have been utilised to formulate breads [4–6]. These breads require a gluten replacement to provide structure and gas retaining properties in the dough [4]. To this end gums are used alone or in combination with non-gluten proteins as egg white or soybean protein or flours [5,6]. Among gums, cellulose derivatives, mainly hydroxypropylmethylcellulose, had proved to increase water absorption and to give softer doughs and breads with improved sensory characteristics. It was found that hydroxypropylmethylcellulose enhanced gas retention in a bread made with rice flour [7,8]. Optimal rice bread formulations were developed using carboxymethylcellulose and hydroxypropylmethylcellulose which met wheat bread reference standards for specific volume, crumb and crust color, firmness and moisture [9].

Surface response methodology has been used with success to analyze the effects of water and gums in objective and sensorial optimization of bread formulas [4,6,9] and dough baking process [10]. The derived equations can describe how test variables affect the response and the interaction among variables, so they are useful to predict the performance of complex systems and to optimise formulations.

Gums affect dough rheological performance, since they mimic the viscoelastic properties of gluten in bread doughs [6,11] and also swelling, gelatinisation, pasting properties and staling of starch. Due to their hydrophilic character they can control the swelling and gelatinisation of starch granules by reducing hydration of the amorphous regions (the trigger

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to gelatinisation) and consequently limiting plasticisation and restricting water to complete the gelatinisation. Thus, higher temperatures are required for gelatinisation [12]. The presence of gums also influences the pasting temperature and the viscosity of the hot starch paste [13,14], the effect being dependent on the structure of the gum. The synergistic interactions observed between some gums and starch during pasting have been ascribed to complex formation between starch components and gums and to phase separation phenomena due to incompatibility between the polysaccharides [9].

Although the gelatinisation of starch in pure aqueous systems is quite well understood, less is known about the transitions in systems like gluten-free bread doughs because of complexity and interactions between starch and ingredients.

The aim of the present study was to determine, by differential scanning calorimetry, the thermal performance of a bread dough consisting of a blend of non allergenic corn and cassava starches with hydroxypropylmethylcellulose (HPMC) as gluten mimetic hydrocolloid in conjunction with egg white. In order to analyse the effects of different components (water, HPMC and egg white) on the thermal transitions of the dough, a Doehlert design [15] and a response surface methodology were used.

2. Experimental

2.1. Materials

Corn (Misky SA, Argentina) and cassava (Indecar SA, Argentina) starches in a 75:25 ratio (dry basis) were selected for dough preparation. The fat, moisture and protein contents of the starches were determined by standard AACC methods 30-10, 44-16, 46-11A [16]. The fat content of both starches was 0.1% (wet basis), the protein content was 0.4 and 0.2% for corn and cassava starches and the moisture content 11.7 and 14.5%, respectively.

HPMC (Methocell E-15 food grade) was obtained from The Dow Chemical Company and dry egg white (EW) from Company Avicola S.A. (Santa Fe, Argentina). Other components were sugar, salt and shortening (Calsa SA, Argentina). Deionised water was used.

2.2. Pasting properties of starches

Pasting properties of corn and cassava starches and the 75:25 blend were determined at 125 rpm (2.083 rps or 2.083 Hz)using a Brabender Viscoamylograph E. The slurries (8 wt.%) at pH 5.5 were heated from 30 to 93 °C at 7.5 °C/min, then they were held at 93 °C for 5 min, afterwards the paste was cooled to 50 °C, and finally kept at 50 °C for 1 min. The initial pasting temperature, the pasting temperature (at maximum viscosity) and maximum viscosity were determined.

Table 1
Gluten-free dough formula

Ingredients	Weight/g (db)	Percent over starch blend[a]
Corn starch	75	
Cassava starch	25	
Sugar		14
Salt		2
Shortening		5
HPMC		0–2
Egg white		0–10
Water		80–110

[a] Corn starch:cassava starch, 75:25.

2.3. Dough preparation

The formula without yeast, adapted from [17], is shown in Table 1 where EW, HPMC, water, salt, sugar and shortening levels are referred to 100 g of the starch blend (dry basis). Dry ingredients were premixed for 2 min in a Philips HR 1456 electric mixer at #1 velocity using curl attachment. Water was added to the ingredients slowly (min) and velocity increased to #3 for 15 min. Dough was covered until use in order to avoid dehydration.

2.4. Differential scanning calorimetry (DSC)

DSC measurements were performed using a Mettler TA 4000 calorimeter with TA72 software using ME 27811 hermetically sealed aluminium capsules of 160 μl with ∼50 mg of dough. To determine the transitions of HPMC and EW, the same mass of solutions of 2 or 10%, respectively, were put into pans. The sample chamber atmosphere was a mix of air and dry nitrogen circulating at 300 ml/min generating a pressure of 1 atm. The calorimeter was calibrated using pure indium as proposed by [18]. An empty aluminium pan was used as reference. A constant scan rate of 10°C/min from 30 to 130 °C was used for determination of the onset (T_o), peak (T_p) and conclusion (T_c) temperatures. The apparent enthalpy ΔH (J/g) was also evaluated. All parameters reported are means of at least two replicates.

2.5. Experimental design

A Doehlert uniform shell design [15] for three factors was selected for the evaluation of the effect of EW, HPMC and water on the thermal transitions of dough. Table 2 shows the coded and real (uncoded) values for variables and their levels. Experiments were randomised and the central point was replicated three times for calculation of the pure error of the methods.

A full quadratic model containing 10 coefficients was used to describe the responses observed to fit the following equation:

$$Y_k = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

Table 2
Real and coded (in brackets) values for the studied variables

Variables	Real[a] and coded values						
Egg white	0 (−0.8165)	5 (0)	10 (0.8165)				
HPMC	0 (−1)	0.5 (−0.5)	1 (0)	1.5 (0.5)	2 (1)		
Water	80 (−0.866)	85 (−0.5774)	90 (−0.2887)	95 (0)	100 (0.2887)	105 (0.5774)	110 (0.866)

[a] Percent over dry starch blend.

where b_0, b_{ii} and b_{ij} are regression coefficients and X_i the coded independent variables, linearly related to EW, HPMC and water levels.

2.6. Statistical analysis

The design was analyzed using the software Statgraphic Plus 3.0 (Manugistic Inc., Rockville, MD, USA) to perform ANOVA, to fit the second-order polynomial equations to data and to generate response surface or contour plots using significant parameters ($P < 0.05$). Residuals were tested for normality, randomness and independence. Coefficients of determination (R^2) were computed and the adequacy of the model was tested by separating the residual sum of squares into pure error and lack of fit ($P > 0.05$). Where the quadratic effects were significant, but linear effects were non-significant, the linear effect, if having $P < 0.1$, was retained [19].

3. Results and discussion

3.1. Thermal transitions of the main individual components of the gluten-free dough

The DSC curves of the basic dough containing the corn and cassava blend (75:25), the shortening, salt and sugar, at 90 and 110% water addition over the starch blend, are shown in Fig. 1. DSC traces of the basic dough at 110% water addition showed a single endotherm. At 90% water addition, the endotherm started to become bimodal. As the amount of water is reduced, the DSC scans show two endotherms [20].

A single endotherm for the basic dough suggested a mixture of the two starches used. This behavior was confirmed by comparing the pasting properties of the starch blend and the individual starches in the absence of the other dough components (Table 3). An almost additive behavior was observed for the onset and pasting temperatures as well as

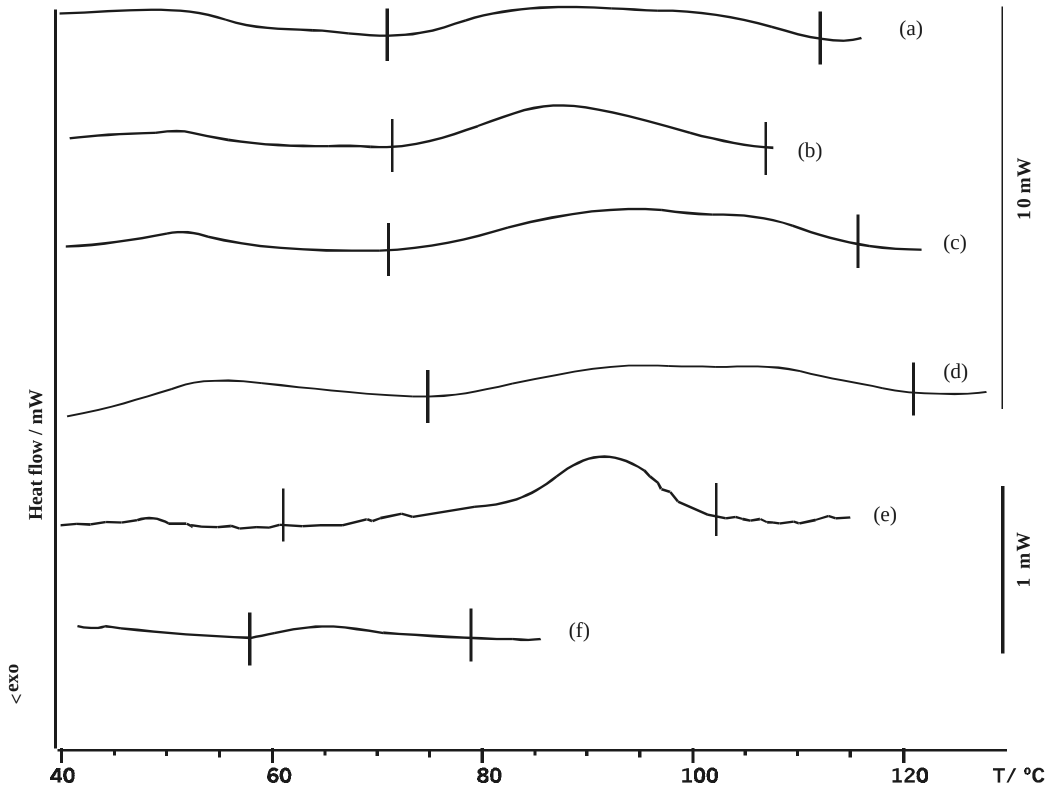

Fig. 1. DSC thermograms of basic dough at: (a) 90% water over the starch blend; (b) 110% water over the starch blend; (c) 90% water and 10% egg white addition over the starch blend; (d) 90% water and 2% HPMC addition over the starch blend; (e) egg white, 10%; and (f) HPMC, 2%.

Table 3
Pasting properties of individual starches and blend

Starch	Initial pasting temperature (°C)	Pasting temperature (°C)	Maximum viscosity/BU
Corn	73.5	89.4	325
Cassava	63.9	73.6	894
Corn:cassava (75:25) Experimental	68.9	88.0	469
Corn:cassava (75:25) Predicted[a]	71.1	85.5	467

[a] Predicted property (P) of the blend $= 0.75\ P_{\text{corn}} + 0.25\ P_{\text{cassava}}$.

Table 5
Experimental matrix and obtained results

Variables			Responses			
HPMC[a]	Water[a]	Egg white[a]	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
1	95	5	67.9	89.6	110.5	9.1
2	95	5	68.3	88.1	110.9	9.1
0	95	5	68.0	86.5	108.4	8.9
1.5	110	5	74.5	84.8	100.7	8.4
0.5	80	5	71.3	93.1	110.7	7.1
1.5	80	5	70.4	92.1	113.8	7.4
0.5	110	5	65.8	85.7	103.5	9.9
1.5	100	10	66.8	86.4	103.7	9.3
0.5	90	0	67.7	86.0	105.4	8.4
1.5	90	0	67.1	85.7	106.0	8.3
1	105	0	67.3	84.1	102.0	8.5
0.5	100	10	66.2	88.2	109.6	9.0
1	85	10	69.2	92.0	113.9	7.1
1	95	5	67.2	88.6	109.9	8.3
1	95	5	67.4	89.0	110.5	9.5

[a] Percent over dry starch blend.

for the viscosity of the paste on attaining 93 °C. In fact the properties for the mixture could be fairly predicted from the properties of the individual components [21]. It was also reported that several starch blends behaved as homogeneous systems. However, at lower water contents competition for water may occur leading to a non-additive behavior [22].

The onset temperatures (T_o), the endothermic peak temperatures (T_p) and the conclusion temperatures (T_c) as well as enthalpy (ΔH) average values for the basic dough at 90% water addition are shown in Table 4. The gelatinisation temperatures are higher than values reported for the starches in water [21,22]. This might be attributed to the increase of gelatinisation temperatures in the presence of salts and sugars [23].

DSC thermogram of egg white in Fig. 1 revealed that the protein denatured over 61–102 °C. The dominant endotherm (T_{p2} in Table 4) at 85.4 °C is due to ovalbumin, present in about 60% by weight. Conalbumin, because of its low thermo-stability and lysozyme appears in the small lower endotherm ($T_{p1} = 72$ °C) [24]. The total apparent enthalpy of EW (6.7 J/g) is lower than that obtained for fresh egg white (23 J/g) indicating a partial denaturation of the proteins during the commercial drying process.

DSC thermogram of HPMC in Fig. 1 showed an endothermic transition over 58.3–79.1 °C, that is attributed to the heat of dehydration [25]. The inverse solubility and gelation behavior of HPMC is well known [26]. When HPMC is exposed to water it undergoes rapid hydration and chain relaxation to form a viscous gelatinous system. On heating HPMC chains dehydrate, the solution becomes less viscous and shows spinodal decomposition. However, before dehydration is complete, polymer–polymer association occurs leading to an increase in viscosity and gel formation. Network formation proceeds because of hydrophobic interactions and competes with phase separation [27]. The enthalpy of dehydration in Table 4 is in accordance with reported values [28].

The addition of the maximum amount of EW (10% over the starch blend) or HPMC (2% over the starch blend) produced a single endotherm (Fig. 1). The absence of an emerging protein peak may reflect the smaller difference in the peak temperatures of the basic dough and EW and also the low EW/starch ratio. In the case of HPMC, it may be mainly attributed to the low HPMC/starch ratio because the difference in the peak temperatures of HPMC and the basic dough was quite large (23 °C**)**.

3.2. *Thermal transitions of the gluten-free dough*

The onset temperatures (T_o), the endothermic peak temperatures (T_p) and the conclusion temperatures (T_c) as well as enthalpy (ΔH) average values for the Doehlert plan in Table 5 showed that maximum variations in T_o, T_p, and T_c were 8.7, 9 and 13.2 °C, respectively. Taking into account that the maximum experimental error in the determination of those temperatures is ±1 °C, it can be concluded that the studied variables significantly affected thermal transition temperatures of the dough. However, the maximum variation in the enthalpy was 2.3 J/g. This difference could be

Table 4
Thermal properties of the basic dough, egg white and HPMC

Component	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Basic dough[a] (90% water over the starch blend)	69	87.4	112	7.1
Egg white (10 g EW + 90 g water)	61	$T_{p1} = 72$, $T_{p2} = 85.4$	102	6.7
HPMC (2 g HPMC + 90 g water)	58.3	64.5	79.1	11.7

[a] Corn starch + cassava starch (75:25), 14% sugar, 2% salt, 5% shortening (percent over dry starch blend).

Table 6
Model coefficients estimated by multiple linear regression and best selected prediction models

Factor	T_o	T_p	T_c
Constant	67.5	89.07	110.3
Linear			
HPMC	1.05*	(−0.17)	(0.02)
Water	−0.70*	−4.18*	−5.93*
EW	(0.01)	2.20*	2.81*
Quadratic			
$HPMC^2$	(0.65)	(−1.74)	(−0.65)
$Water^2$	3.78*	(0.26)	−4.02*
EW^2	(−1.24)	−2.58*	−4.13*
Interactions			
HPMC × water	5.54*	(0.19)	−3.52*
HPMC × EW	(−1.28)	(−0.85)	−2.73*
Water × EW	(−0.73)	(−1.30)	−3.70*
R^2	0.83	0.96	0.96
Lack of fit	*	NS	*

The symbol (*) indicates ($P < 0.05$) and numerals in parenthesis show non-significant values. Reduced equations for thermal transitions of dough (coded values); $T_o = 67.5 + 1.05\ \text{HPMC} - 0.70\,\text{water} + 5.54\,\text{HPMC} \times \text{water} + 3.78\ \text{water}^2$; $T_p = 89.07 - 4.18\,\text{water} + 2.20\,\text{EW} - 2.58\ \text{EW}^2$; $T_c = 110.3 - 5.93\,\text{water} + 2.81\,\text{EW} - 3.52\,\text{HPMC} \times \text{water} - 2.73\,\text{HPMC} \times \text{EW} - 3.70\,\text{water} \times \text{EW} - 4.02\,\text{water}^2 - 4.13\ \text{EW}^2$.

considered non-significant because the experimental error in enthalpy determinations was ±1 J/g.

Estimated regression coefficients for each transition temperature were obtained from responses by multiple linear regression analysis (Table 6) and best explanatory equations are also shown.

In all cases R^2 coefficients were high. For T_o the model was able to explain more than 80% of the observed responses and for T_p, and T_c 96%. For T_o, and T_c the lack of fit test was significant which means that the order of the regression was not secondary (the model may not have included all appropriate functions of independent variables or the experimental region may be to large for a quadratic model was used). It was pointed out that when a large amount of data were included in the analysis, a model with significant lack of fit could still be used [29]. We considered the high coefficients R^2 as evidence of the applicability of the regression model between the range of variables included.

3.2.1. Onset temperature

Significant regression coefficients for T_o showed that EW did not affect the onset temperature. HPMC had only linear effects and water had linear and quadratic effects. Interactions on T_o were observed between the water and HPMC and the largest value of estimated coefficient for the interaction term ($b = 5.18$) indicated that it was the most important variable influencing T_o. The variable which had only linear effect was HPMC ($b = 1.05$) and its positive value indicates that T_o increased with increasing HPMC. When non-starch polysaccharides are present they have the capacity to hydrate and consequently restrict the mobility of water and hence delay initiation of the gelatinisation process. A small increase on the onset temperature of wheat flour gelatinisation in the presence of HPMC has been reported by [14]. The presence of other non-starch polysaccharides in general terms elevate gelatinisation temperatures [12].

The negative value of the linear effect for water indicates that T_o decreased with increasing water content. The positive quadratic term for water showed the existence of a minimum for the decrease of T_o as a function of water content. Starch gelatinisation temperatures increase when water is not sufficient for full plasticization of starch. If the amount of water available to the starch is below 30–50%, the amount of water controls the gelatinisation temperature of the starch [30,31].

Contour plots and response surface for T_o were obtained with the reduced regression model and plotted in Fig. 2(a and b) as a function of water and HPMC levels in the dough, with the EW variable fixed at the central point (EW = 5%). Similar plots were obtained at other constant EW levels as this variable did not influence T_o. The response surface showed a saddle point which indicated the absence of a unique maximum or minimum. This type of response provides an advantage to dough processing since a broad range of water and HPMC concentrations could be selected to generate a desired onset of gelatinisation. It is interesting to note that a broad range of combined levels of HPMC and water gave the lowest onset gelatinisation temperatures. Nevertheless, the highest onset temperatures were obtained at the highest HPMC and water levels or at the lowest levels for both variables.

The contour lines indicated that the influence of water on T_o was dependent on HPMC concentration. For low values of HPMC, the effect of increasing water content was to decrease the onset of starch gelatinisation as it has been reported for starch systems of reduced water content [30]. Therefore, it may be concluded that at HPMC levels below 1% would prevail the plasticizing effect of water on starch. At higher HPMC levels (>1%), the increase of water first lowered T_o but then increased it, so that maximum values (≈75 °C) were reached at the highest water and HPMC levels.

Similarly, the effect of HPMC on T_o depended on the water level. At low water levels (<95%) the initiation of gelatinisation occurred at lower temperatures as the level of HPMC was increased from 0 to 2%. A progressively decrease of the amylogram gelatinisation temperature of starch was found as the amount of guar gum increased [32]. A similar effect has been found [14,33] which reported an earlier beginning of gelatinisation of starch caused by xanthan, κ-carrageenan and alginate. The decrease in gelatinisation temperatures caused by non-starch polysaccharides has been attributed to interactions between starch and the hydroxyl groups of the hydrocolloids [33]. On the contrary, at water levels above 95% the addition of HPMC delayed the initiation of starch gelatinisation. It was also found that HPMC increased the pasting temperature of starch [14].

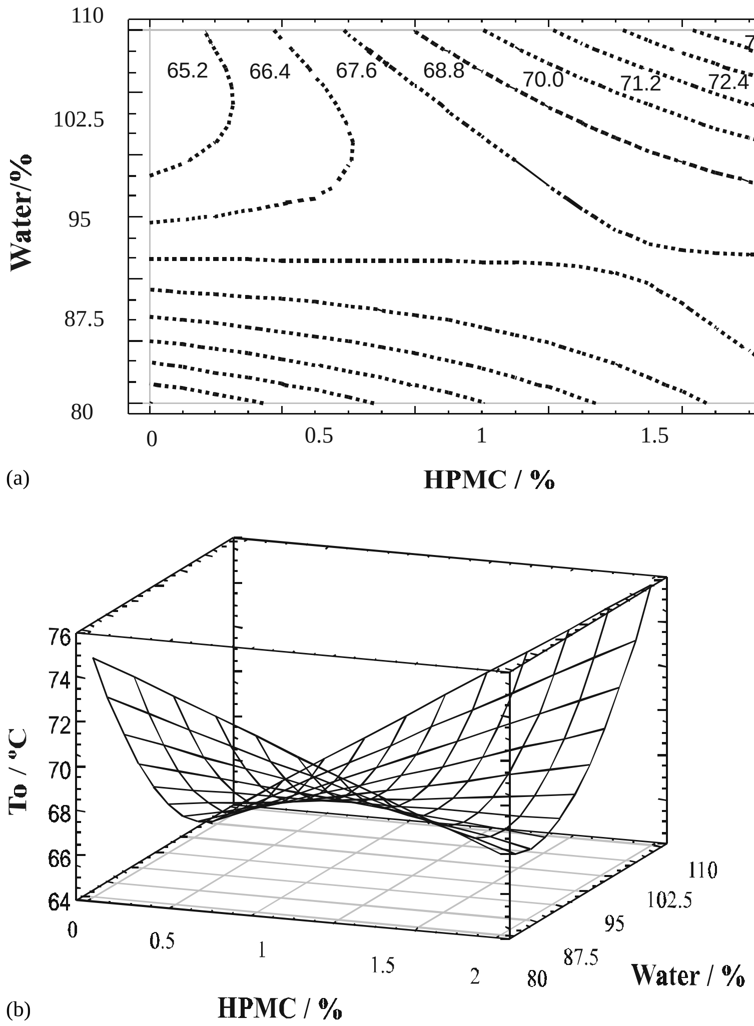

Fig. 2. Contour (a) and response surface plots (b) for the onset temperature.

The above results show that non-starch polysaccharides may cause starch to resist gelatinisation or to gelatinise earlier, being the resultant effect dependent on water availability and hydrocolloid concentration but more on the interactions between those factors.

The complex behavior of HPMC is difficult to comprehend and factors like competition for water between the components and phase separation between incompatible biopolymers further complicates the view. Nevertheless, the performance of HPMC on heating the dough may be interpreted on the basis of the inverse solubility shown by this polysaccharide, that involves (1) chains dehydration on heating causing a decrease in viscosity and then (2) polymer–polymer association/gel formation with a concomitant increase in viscosity.

It may be speculated that in conditions of higher water availability (>95%), hydrated HPMC present in the continuous phase surrounding starch granules could undergo dehydration and further association/gelation in the temperature range 58–79 °C (Table 4) that is close to the onset of starch gelatinisation. In fact, a 2 wt.% HPMC solution completely gelled at 63 °C (visual assessment). Gelation of HPMC, could promote phase separation between starch and HPMC gel with the consequence of restricting swelling of starch granules and delaying the initiation of gelatinisation because gel phase might be expected to limit water migration from one granule to another. A restriction of the gelatinisation of starch granules was reported when embebbed in a gel matrix of gellan or gellan-locust bean gum [22].

In a more restricted water environment (<95%), that also means a more viscous system with lower mobility, HPMC could undergo dehydration close to the onset temperature of starch gelatinisation, but could not associate to gel so that the swelling and gelatinisation of starch would not be restricted by a gelled continuous phase. In addition, possible interactions of hydroxyl groups of HPMC with starch could further contribute to facilitate gelatinisation. It was shown [34] that noticeable changes in the association/precipitation behavior of hydroxypropylcellulose occurs in the presence of starch granules. They proposed that HPC coats the surface of the starch granules in preference to forming a precipitate. At HPC levels greater than a critical ratio, free HPC precipitates upon heating.

3.2.2. Peak and conclusion temperatures

Regression coefficients for T_p and T_c showed that HPMC did not affect the peak and conclusion temperatures. This is consistent with the view that HPMC transitions occur close to the onset of starch gelatinisation.

The reduced regression models for both transition temperatures were similar. Water had only linear effects on T_p but showed also quadratic effects on T_c. The negative value of the linear term indicated the plasticizing effect of water on starch components. This factor showed the highest regression coefficient indicating that water was the variable that more affected T_p and T_c.

EW showed a positive linear effect and a negative quadratic effect indicating that the increase of EW concentration increases both transition temperatures up to a maximum. Interaction effects were no significant.

Peak temperatures for the protein and starch occurred in the same temperature range (Table 4) so that denaturation and gelation of EW would compete with the completion of starch gelatinisation. On gelling, EW would immobilize a part of the water, markedly reducing water availability for starch gelatinisation. The increased viscosity of the continuous gelled phase where starch granules are included would additionally hinder the migration of water between starch granules. Similar effects of egg white on properties of baked products have been reported by [5,34]. Corn starch gelatinisation temperatures were also shifted to higher values in the presence of cowpea protein [35].

Contour plots and response surface for T_p and T_c were obtained with the reduced models and plotted in Figs. 3(a and b) and 4(a and b) as a function of water and EW levels in the dough, with the HPMC variable fixed at the central point (HPMC = 1%). Similar plots were obtained at other constant HPMC levels as this variable did not influence T_p and T_c.

The contour lines indicated an additive response for the variables influencing T_p and T_c. Maximum T_p and T_c (92 and 112 °C, respectively) were found in doughs with the lowest level of water and the highest level of EW. On the contrary, doughs with the highest level of water and small amount or none EW showed minimum T_p and T_c (84 and 100 °C).

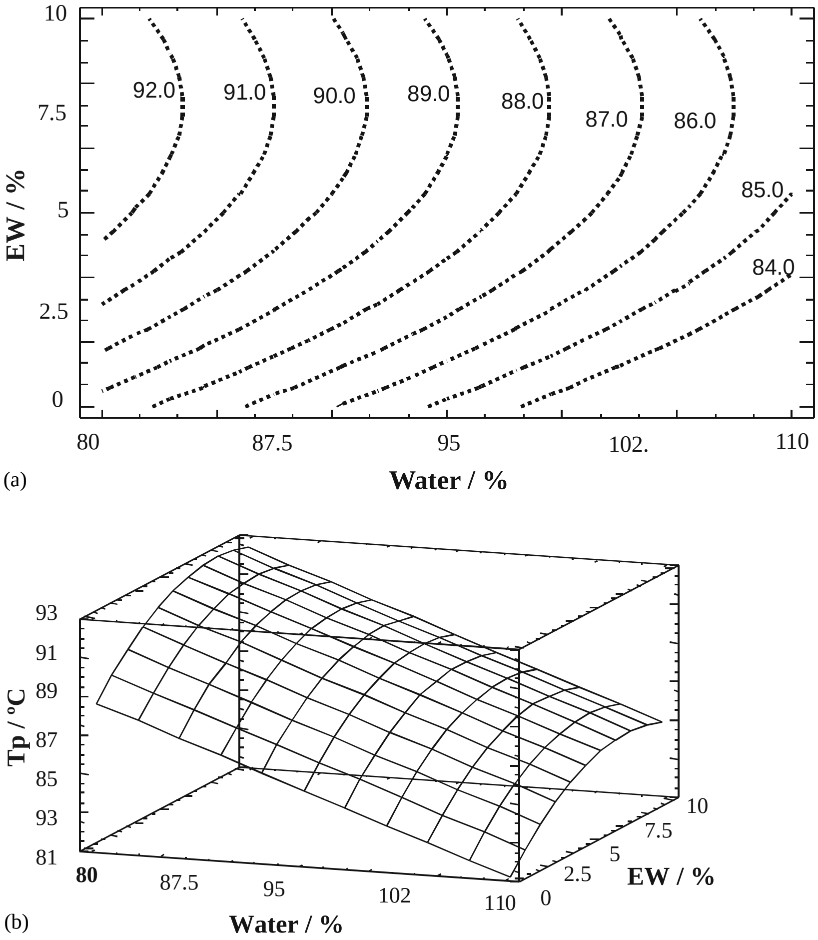

Fig. 3. Contour (a) and response surface plots (b) for the peak temperature.

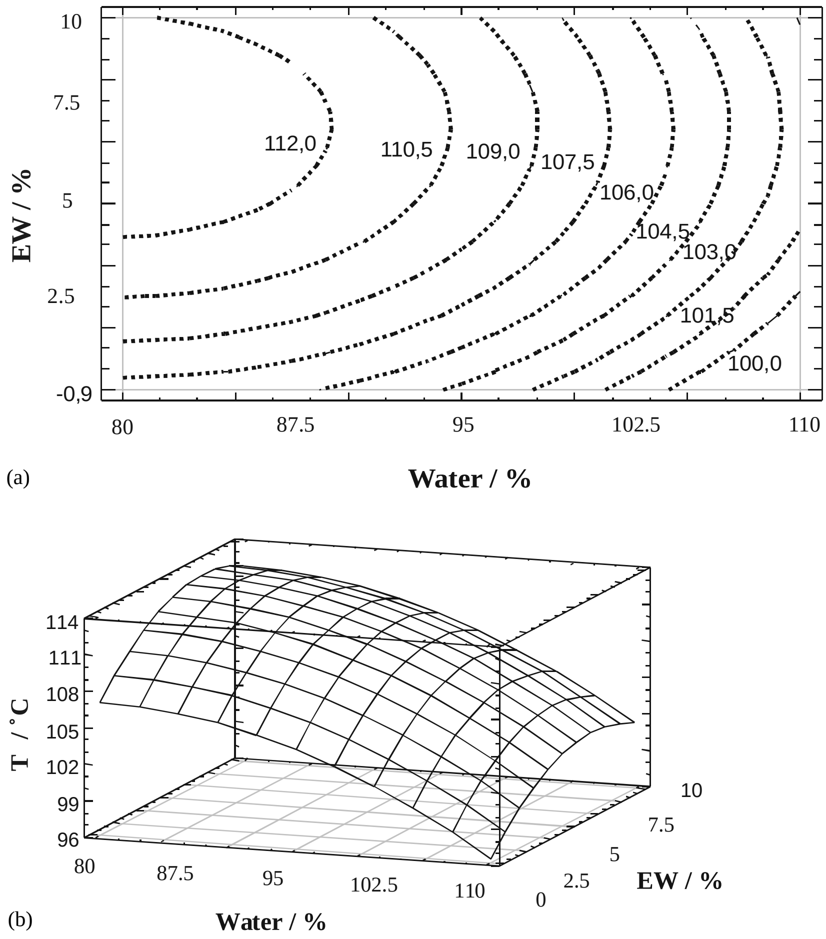

Fig. 4. Contour (a) and response surface plots (b) for the conclusion temperature.

4. Conclusion

Response surface methodology could be successfully applied to a complex system such as a gluten-free dough to analyze the effect of each one of the components on the thermal behavior of the dough and to reveal interactions between them.

The level of water, HPMC and EW addition to the dough greatly influenced the transition temperatures. HPMC–water interactions mainly controlled the onset temperature of starch gelatinisation. On the other hand, the peak and conclusion temperatures were determined by the additive and opposite effects of water and EW.

The results allow to find several optimum combinations of HPMC, water and EW that could result in doughs with a desired thermal performance. For instance when looking for a dough that starts to gelatinise and also proceeds and concludes the gelatinisation at the lowest temperatures, an intermediate level of HPMC (1%) and EW (5%) should be used in combination with a water level of approximately 100%.

On the contrary, low water (80%) and HPMC ($<0.5\%$) levels in combination with a high EW level (10%), would give rise both the onset, peak and conclusion temperatures of gelatinisation.

Moreover, it could be desirable that the dough starts to gelatinise early but then the starch granules resist to fully gelatinise during baking. This specific thermal performance could be met by selecting a low water level (80–90%) in combination with a high HPMC (1.5–2%) and EW (5–10%) levels. In this dough formulation the difference between the onset and peak gelatinisation temperatures would be as large as 27 °C.

Anyway, sensorial and objective measurements of the bread quality would be performed to determine the optimum formulation.

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