# Modelling the effects of psyllium and water on dough parameters using Mixolab® and their relationship with physical properties and acceptability of gluten-free bread[1]

## Análise dos efeitos do psyllium e da água nos parâmetros da massa no Mixolab® e sua relação com as propriedades físicas e aceitabilidade de pães sem glúten

## Análisis de los efectos del psyllium y del agua sobre los parámetros de la masa en Mixolab® su relación con las propiedades físicas y aceptabilidad de los panes sin gluten

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**Abstract**

This study aimed to investigate the effects of psyllium (P) and water (W) on dough Mixolab® parameters, and their relationship with gluten-free bread (GFB) physical properties and acceptability. A $2^2$ factorial design with three center points was used, in which P levels ranged from 2.86 to 17.14% and W levels from 82.14 to 117.86% on a flour basis. Samples were

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compared to a control GFB (0P:100W), and data were evaluated using regression models and multiple factor analysis (MFA). The predicted model equations were significant ($R^2_{adj}$= 82-99%, p<0.05) and showed that P increased dough consistency (C1), protein weakening (C2), gelatinization (C3), stability (C4) and retrogradation (C5) of starch, whereas W or its interaction with P decreased these parameters. MFA's three dimensions explain 94.86% of the total variation. Factor 1 (57.02%) positively discriminates the loaf-specific volume and all acceptability attributes, but negatively discriminates crumb firmness and C1, C2, C3, C4, and C3-C4 Mixolab parameters, especially in the 2.86P:82.14W sample. Factor 2 (26.30%) positively discriminates the C5, C1-C2, and C5-C4 Mixolab parameters and central points of the study, but negatively discriminates the control GFB. Factor 3 (11.54%) positively discriminates crumb moisture and 2.86P:117.86W and 17.14P:117.86W samples, unlike 2.86P:82.14W, which is negatively discriminated. We found results regarding dough Mixolab parameters to explain P and W influence and its capability of predicting GFB physical properties and acceptability.

**Keywords:** Bread quality; Mixolab; Multiple factor analysis.

**Resumo**

Este estudo objetivou investigar os efeitos do psyllium (P) e água (A) nos parâmetros da massa no Mixolab® e sua relação com as propriedades físicas e aceitabilidade de pães sem glúten (PSG). Um planejamento fatorial 2² com três pontos centrais foi usado, cujos níveis de P (2,86 a 17,14%) e A (82,14 a 117,86%) variaram na base farinha. As amostras foram comparadas à PSG controle (0P:100A). Os dados foram avaliados pelos modelos de regressão e análise fatorial múltipla (AFM). As equações do modelo previsto foram significativas ($R^2_{aj}$ = 82-99%, p < 0,05) e mostraram que P aumentou a consistência da massa (C1), enfraquecimento da proteína (C2), gelatinização (C3), estabilidade (C4) e retrogradação (C5) do amido, enquanto A, ou sua interação com P diminuíram esses parâmetros. As três dimensões da AFM explicam 94,86% da variação total. O fator 1 (57,02%) discrimina positivamente o volume específico do pão e todos os atributos de aceitabilidade, enquanto a firmeza do miolo e os parâmetros C1, C2, C3, C4 e C3-C4 do Mixolab foram discriminados negativamente, destacando-se a amostra 2,86P:82,14A. O fator 2 (26,30%) discrimina positivamente os parâmetros C5, C1-C2 e C5-C4 do Mixolab e os pontos centrais do estudo e discrimina negativamente PSG controle. O fator 3 (11,54%) discriminou positivamente a umidade do miolo e as amostras 2,86P:117,86A e 17,14P:117,86A; enquanto discriminou negativamente a amostra 2.86P:82.14A. Em conclusão, os parâmetros da massa no Mixolab explicam a influência de P e A e indicam seu potencial em predizer as propriedades físicas e aceitabilidade de PSG.

**Palavras-chave:** Qualidade do pão; Mixolab; Análise fatorial múltipla.

**Resumen**

Este estudio investigó los efectos del psyllium (P) y del agua (A) sobre los parámetros de la masa en Mixolab® y su relación con las propiedades físicas y aceptabilidad de panes sin gluten (PSG). Se utilizó un diseño factorial 2² con tres puntos centrales, cuyos niveles de P (2.86 a 17.14%) y A (82.14 a 117.86%) variaron en la base de la harina. Las muestras se compararon con PSG controles (0P:100A). Se evaluaron los datos mediante los modelos de regresión y análisis factorial múltiple (AFM). Las ecuaciones del modelo predicho fueron significativas ($R^2_{aj}$=82-99%, p<0.05) y señalaron que P aumentó la consistencia de la masa (C1), proteína debilitada (C2), gelatinización (C3), estabilidad (C4) y retrogradación del almidón (C5), mientras que A, o su interacción con P, disminuyó esos parámetros. Las tres dimensiones de la AFM explican 94,86% de la variación total. El factor 1 (57,02%) discrimina positivamente el volumen específico del pan y todos los atributos de aceptabilidad, mientras que la firmeza de la miga y los parámetros C1, C2, C3, C4 y C3-C4 fueron discriminados negativamente, destacándose la muestra 2.86P:82.14A. El factor 2 (26,30%) discrimina positivamente los parámetros C5, C1-C2 y C5-C4 y los puntos centrales del estudio y discrimina negativamente PSG controle. El factor 3 (11,54%) discriminó positivamente la humedad de la miga y las muestras 2.86P:117.86A y 17.14P:117.86A; mientras que discriminó negativamente la muestra 2.86P:82.14A. En conclusión, los parámetros de la masa en Mixolab explican la influencia de P y A e indican su potencial para predecir las propiedades físicas y aceptabilidad de PSG.

**Palabras clave:** Calidad del pan, Mixolab; Análisis factorial múltiple.

## 1. Introduction

The increasing demand for high-quality gluten-free bread (GFB) poses a major challenge for food scientist, chefs, bakers, and the food industry due to the growing number of individuals with or without gluten intolerance following a gluten-free diet worldwide (Capriles et al., 2020).

GFB is often perceived as a product with unpleasing appearance and poor texture, mouthfeel, and taste, besides being known for its poor nutritional quality and short shelf-life, limitedly available and significantly more expensive than wheat bread. Consequently, numerous studies focused on developing and improving the quality of GFB, as indicated by literature reviews (Bender & Schönlechner, 2020; Capriles et al., 2020).

Given that GFB quality (especially its texture) is compromised by the lack of viscoelastic network in its dough, it is necessary to design matrices to meet breadmaking requirements. In this sense, and in view of the growing demand for functional foods beneficial to health, the psyllium (P) – a soluble fiber obtained from the seed husks of *Plantago ovata* Forsk – has aroused food scientist's interest due to its role in helping intestinal transit, cholesterol, blood glucose, and satiety control (Belorio & Gómez, 2020; Franco et al., 2020). When hydrated, P has important technological properties in food application, particularly its solubility and high-water binding and retention capacity, resulting in thickening and gel formation (Pejcz et al., 2018; Yu et al., 2017).

Recent studies showed that incorporating P into the dough may increase its viscosity and gas holding capacity, and improve GFB volume, structure, texture, appearance, acceptability, and shelf-life (Cappa et al., 2013; Fratelli et al., 2018; Mancebo et al., 2015; Santos et al., 2020; Ziemichód et al., 2019), besides increasing its fiber content and reducing its glycemic response (Fratelli et al., 2018). However, the impact of P on gluten-free dough and bread properties depends on its added levels, water content, and other ingredients present in the formulation (Cappa et al. 2013; Mancebo et al. 2015; Fratelli et al. 2018). Studies addressing how gluten-free dough affects GFB properties, especially with the incorporation of P, are still scarce.

Mixolab® is a device that has been successfully used to assess wheat dough systems under similar breadmaking conditions (Rosell et al., 2007), providing a thoroughly rheological analysis; it is also applicable to gluten-free systems (Matos & Rosell, 2013). However, the relationship between gluten-free dough rheology and GFB properties using P is still little explored, requiring further research.

Our previous work reported P as a promising ingredient to improve GFB physical, sensory, and nutritional properties concomitantly (Fratelli et al, 2018). Considering that, we further investigated the effects of P and water on dough Mixolab® parameters and their relationship with GFB physical properties and acceptability.

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## 2. Methodology

This research is a lab study of quantitative nature, of which was relationed the dough and bread properties.

Rice flour (Urbano Agroindustrial Ltda.) and cassava starch (General Mill Brasil Alimentos Ltda.), purchased from local Brazilian stores, psyllium ( P) (VITACEL® Psyllium P95), donated by JRS Latinoamericana Ltda, and water were the materials employed in this study.

A full $2^2$ factorial design was adopted in four trials, in which P levels ranged from 2.86 to 17.14% and W levels from 82.14 to 117.86%, and the three-center points repetition contained 10% P and 100% W on a flour weight basis (75% rice flour and 25% cassava starch). These trials were compared to a control (0P:100W), totalling eight dough samples.

The doughs were analyzed using the Chopin+ protocol in Mixolab®2 (Chopin Technologies, France) and the American Association of Cereal Chemists (AACC) 54-60.01 method (2010). Levels of P and W were defined according to the factorial design. The following parameters were considered (torques in Nm) in the analysis: initial consistency (C1), protein weakening (C2), gelatinization (C3), gel stability (C4), and retrogradation (C5) of starch; and the secondary – C1(at 8min)-C2, C3-C2, C3-C4 and C5-C4, obtained by the differences between primary parameters regarding the rates of protein weakening, gelatinization, hydrolysis, and retrogradation of starch, respectively (Matos & Rosell, 2013).

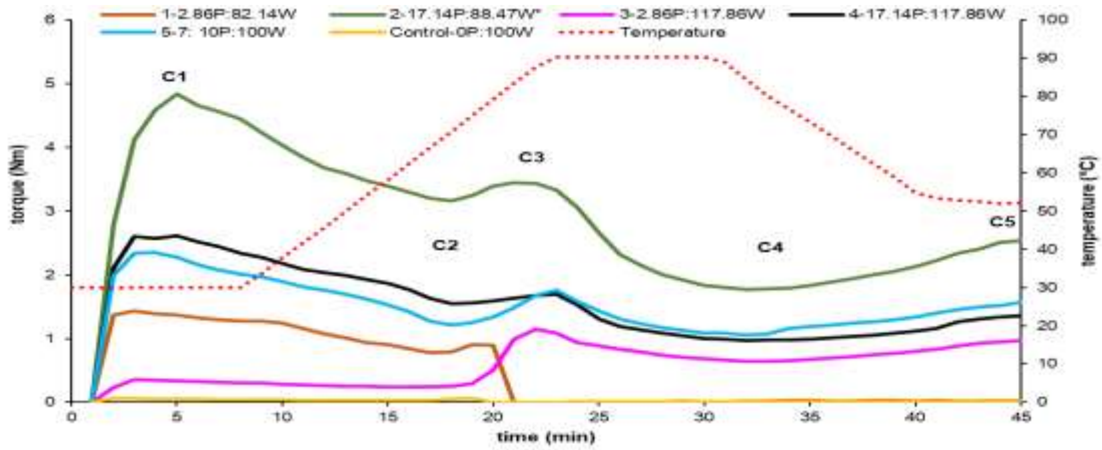
The dough Mixolab® thermomechanical properties were related to the physical properties (loaf-specific volume, crumb moisture, and firmness) and acceptability (appearance, color, aroma, texture, flavor, and overall liking evaluated by 53 consumers) of the GFB developed and studied by Fratelli et al. (2018).

Data were compared using one-way ANOVA at $p < 0.05$ and Tukey's test, and evaluated using regression models considering adjusted $R^2 \geq 70\%$ and $p < 0.05$. These analyses were performed using Statistica 13.5 software (Tibco Inc., USA, 2018), and the relationships between doughs and bread properties were verified considering the multiple factor analysis (MFA) using XLSTAT 2020.2 software (Addinsoft, USA, 2020).

## 3. Results and Discussion

Figure 1 shows the dough thermomechanical curves, and in the sequence, Table 1 presents these parameters values.

**Figure 1.** Curves obtained in Mixolab® for evaluating the effects of different psyllium (P) and water (W) levels on gluten-free bread doughs.




* water-level adjusted to the initial design to enable the analysis. Source: Authors.

**Table 1.** Mixolab® parameters of gluten-free bread doughs with different psyllium (P) and water (W) levels.

| Trials | Torque (Nm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | C1 | C2 | C3 | C4 | C5 | C1-C2 | C3-C2 | C3-C4 | C5-C4 |
| 1- 2.86P:82.14W | 1.46$^{d}$ ±0.03 | 0.79$^{d}$ ±0.02 | 0.99$^{d}$ ±0.00 | 0.00$^{e}$ ±0.00 | 0.03$^{d}$ ±0.02 | 0,49$^{c}$ ±0.00 | 0.20$^{d}$ ±0.02 | 0.99$^{b}$ ±0.01 | 0.03$^{d}$ ±0.02 |
| 2-17.14P:88.47W* | 4.86$^{a}$ ±0.02 | 3.14$^{a}$ ±0.03 | 3.52$^{a}$ ±0.06 | 1.76$^{a}$ ±0.00 | 2.58$^{a}$ ±0.00 | 1,06$^{a}$ ±0.01 | 0.37$^{c}$ ±0.03 | 1.75$^{a}$ ±0.05 | 0.82$^{a}$ ±0.00 |
| 3-2.86P:117.86W | 0.36$^{e}$ ±0.00 | 0.23$^{e}$ ±0.01 | 1.15$^{c}$ ±0.02 | 0.63$^{d}$ ±0.00 | 1.05$^{c}$ ±0.02 | 0,07$^{d}$ ±0.01 | 0.92$^{a}$ ±0.01 | 0.51$^{d}$ ±0.01 | 0.42$^{c}$ ±0.02 |
| 4-17.14P:117.86W | 2.64$^{b}$ ±0.01 | 1.53$^{b}$ ±0.00 | 1.71$^{b}$ ±0.00 | 0.99$^{c}$ ±0.04 | 1.59$^{b}$ ±0.10 | 0,75$^{b}$ ±0.00 | 0.18$^{d}$ ±0.00 | 0.72$^{c}$ ±0.03 | 0.59$^{bc}$ ±0.06 |
| 5-10P:100W | 2.36$^{c}$ ±0.06 | 1.18$^{c}$ ±0.00 | 1.73$^{b}$ ±0.02 | 1.04$^{bc}$ ±0.01 | 1.72$^{b}$ ±0.02 | 0,78$^{b}$ ±0.03 | 0.56$^{b}$ ±0.01 | 0.69$^{c}$ ±0.03 | 0.68$^{b}$ ±0.03 |
| 6-10P:100W | 2.33$^{c}$ ±0.06 | 1.16$^{c}$ ±0.07 | 1.73$^{b}$ ±0.04 | 1.07$^{b}$ ±0.03 | 1.71$^{b}$ ±0.04 | 0,76$^{b}$ ±0.00 | 0.58$^{b}$ ±0.03 | 0.66$^{c}$ ±0.07 | 0.63$^{b}$ ±0.07 |
| 7-10P:100W | 2.36$^{c}$ ±0.02 | 1.18$^{c}$ ±0.00 | 1.76$^{b}$ ±0.00 | 1.09$^{b}$ ±0.01 | 1.71$^{b}$ ±0.07 | 0,76$^{b}$ ±0.02 | 0.58$^{b}$ ±0.02 | 0.67$^{c}$ ±0.01 | 0.62$^{b}$ ±0.08 |
| Control-0P:100W | 0.11$^{f}$ ±0.00 | 0.04$^{f}$ ±0.02 | 0.07$^{e}$ ±0.02 | 0.00$^{e}$ ±0.00 | 0.00$^{d}$ ±0.00 | 0,02$^{d}$ ±0.01 | 0.03$^{e}$ ±0.00 | 0.07$^{e}$ ±0.02 | 0.00$^{d}$ ±0.00 |

Values are mean ± standard deviation. Different letters in the same column are different significantly (Tukey test $p$ <0.05).
* water-level adjusted to the initial design to enable the analysis. Source: Authors.

The combination of high P and low W levels found in trial 2 impair the analysis given the high force exerted on the device. The W level was thus increased to the minimum required value to enable the analysis. The highest peaks in the main Mixolab parameters were observed in trial 2, whereas the control trial (0P:100W) showed the lowest peaks (Figure 1 and Table 1).

C1 dough consistency increased the most with the growing P addition, whereas W affected this parameter the least. In this study, the increasing P contribution reduced C2 points (Figure 1) and is in agreement with the observations by Pejcz et al. (2018) on the wheat dough with added P (4 and 8%). In the same way, the C1-C2 rate increased due to P addition but only differs between the samples with low P concentration (trial 3-2.86P:117.86W) and control (0P:100W) (Table 1). However, Santos et al. (2020) observed torque reduction in C2 at the highest P levels (12.5%) combined with the highest levels of chickpea flour (100%). According to these authors, high P levels are highly capable of forming complexes with system proteins through both ionic and nonionic interactions, thus affecting dough strength (Santos et al., 2020).

The dough properties with a potential tendency of relations with bread characteristics were characterized by points C3 to C5 (Figure1, Table 1). Different P and W levels resulted in different doughs consistency in these stages. Compared to the control, incorporating P into the dough significantly increased consistency at C3 (from 14 to 50 times), which is more evident in trial 3 for presenting the highest concentration. However, we observed no difference between control and trial 1, with the lowest P and W levels, regarding C4 and C5 (Figure 1, Table 1). According to Pejcz et al. (2018), P is capable of absorbing forty times its weight in water, which could strongly affect dough functional and technological properties, including rheology, as it would limit the W content available to starch hydration. Incorporating P into the dough increased starch gelatinization susceptibility (C3-C2), amylase activity (C4-C3), and retrogradation tendency (C5-C4). These parameters may influence bread quality, especially staling kinetics during storage (Rosell et al., 2007). In general, the observed changes in parameters may be explained by the interaction between starch and different incorporated P levels competing for the available W content on doughs systems.

Based on these results and statistical calculation, we adjusted the trial 2 to real levels of factorial design.

Table 2 shows prediction equations for the experimental model.

**Table 2.** Predicted model equations indicating psyllium and water effects [a] and their interaction on the Mixolab® properties of gluten-free dough.

| **Parameters** | **Predicted model equations** | **$R^2_{adj}$ (%)** [b] | ***p-value*** [c] |
|---|---|---|---|
| C1 | $Y_a = 2.40 + 1.53x_1 - 0.93x_2 - 0.38x_1x_2$ | 99.79 | 0.000 |
| C2 | $Y_b = 1.27 + 0.84x_1 - 0.47x_2$ | 92.42 | 0.000 |
| C3 | $Y_c = 1.84 + 0.84x_1 - 0.48x_2 - 0.56x_1x_2$ | 98.07 | 0.000 |
| C4 | $Y_d = 0.98 + 0.60x_1 - 0.41x_1x_2$ | 94.38 | 0.000 |
| C5 | $Y_e = 1.54 + 0.87x_1 - 0.60x_1x_2$ | 93.90 | 0.000 |
| C1-C2 | $Y_f = 0.67 + 0.31x_1 - 0.18x_2 + 0.03\, x_1x_2$ | 81.95 | 0.000 |
| C3-C2 | $Y_g = 0.50 - 0.11x_1 + 0.10x_2 - 0.26\, x_1x_2$ | 86.17 | 0.000 |
| C3-C4 | $Y_h = 0.86 + 0.24x_1 - 0.38x_2 - 0.14\, x_1x_2$ | 66.83 | 0.000 |
| C5-C4 | $Y_i = 0.53 + 0.22x_1 + 0.07x_2 - 0.13\, x_1x_2$ | 56.82 | 0.000 |

[a] $x_1$= psyllium. $x_2$= water. [b] $R^2_{adj}$= adjusted coefficient of determination [c] *p:* probability level < 0.05 level. Source: Authors.

Based on Table 2 results, we may verify that P increased all primary parameters (from C1 to C5) evaluated by Mixolab, while W or its interaction with P decreased them. In turn, P, W, or their interaction, caused the opposite effect on secondary parameters. Most parameters adjusted to models ($R^2_{adj}$= 82-99%, $p$=0.000), but not C3-C4 and C5-C4 ($R^2_{adj}$ < 70%).

Our findings corroborate those reported by Aprodu & Banu (2015), who verified that dough parameters varied with different hydration levels due to starch and fiber competition for water. Incorporating P into a dough of low water level increases C4 and C5; in turn, incorporating it into a dough of high W level decreases C3 and C5.

Figure 2 shows the relationships between dough and bread properties, whereby MFA three dimensions explain 94.86% of the total variation.

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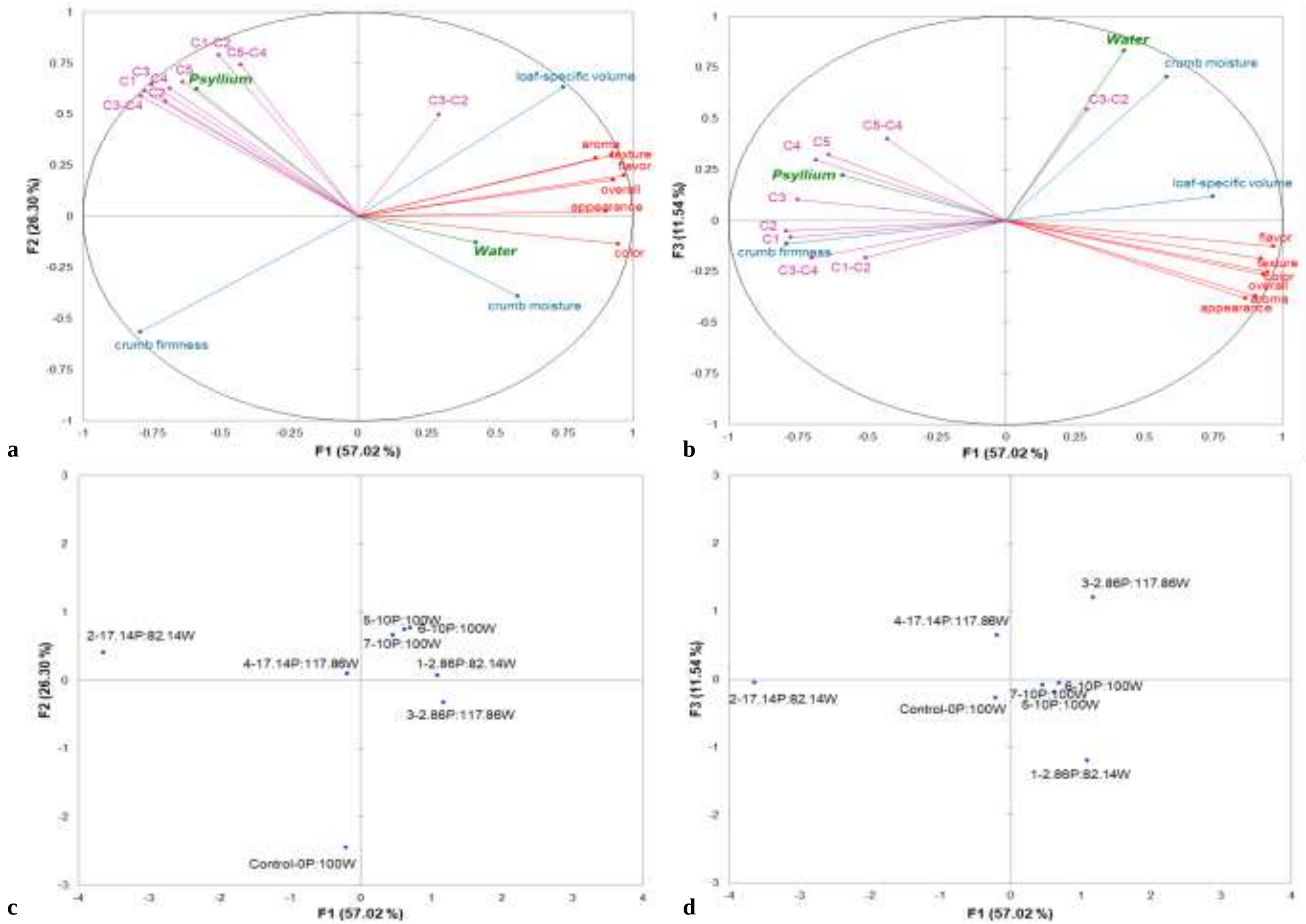
**Figure 2**. Multiple factor analysis correlating dough parameters with physical and sensorial properties of the evaluated gluten-free breads.

(a-b) Map of dough Mixolab® parameters (in pink), bread physical (in blue) and sensorial (in red) properties, and ingredients as supplementary variables (in green).

(c-d) Map of gluten-free bread coded by different psyllium (P) and water (W) levels




Source: Authors.

Factor 1 (57.02%) positively discriminates loaf-specific volume and all sensory acceptability attributes. However, negatively discriminates crumb firmness and C1, C2, C3, C4 and C3-C4 Mixolab parameters, especially in the 17.14P:82.14W sample, due to its higher P level. Factor 2 (26.30%) positively discriminates C5, C1-C2, and C5-C4 Mixolab parameters and central points of the study, but negatively discriminates the control GFB. Factor 3 (11.54%) positively discriminates crumb moisture due to higher W levels, explaining 2.86P:117.86W and 17.14P:117.86W samples, but negatively discriminates the 2.86P:82.14W sample.

Low or intermediate levels of P and W (particularly the samples 2.86P:82.14W and 10P:100W – Figures 2c, 2d) present dough parameters axes opposite to those of bread properties. This finding indicates a favorable dough consistency for GFB with greater loaf-specific volume, as well as greater sensory acceptability of all evaluated attributes, observed by the proximity between these axes and lower crumb firmness (Figures 2a, 2b).

Our finding corroborates those reported by our previous studies (Fratelli et al., 2018), as the presence of P may positively influence crumb softness if the dough is properly hydrated. Conversely, deficient hydration decreases loaf volume, crumb softness, and sensory acceptability.

## 4. Conclusion

We found balanced P and W levels to strongly affect dough parameters, resulting in GFB with greater loaf-specific volume, crumb softness, and sensory acceptability for the evaluated attributes.

Mixolab dough parameters explain P and W influence and indicate its potential for predicting GFB physical properties and acceptability, and that it could be helpful in guiding further studies in both gluten-containing and gluten-free breadmaking.

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