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Optimization of gluten-free sponge cake fortified with whey protein concentrate using mixture design methodology

Imène Ammar [a,*], Houda Gharsallah [a,c], Abir Ben Brahim [a], Hamadi Attia [a], M.A. Ayadi [a], Bilel Hadrich [b], Imène Felfoul [a,*]

[a] Université de Sfax, ENIS, Laboratoire Analyse, Valorisation et Sécurité des Aliments, Sfax 3038, Tunisia
[b] Laboratory of Enzyme Engineering and Microbiology, Engineering National School of Sfax (ENIS), University of Sfax, Tunisia
[c] Tunisian Olive Institute, University of Sfax, Sfax, Tunisia

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ABSTRACT

This study aimed to optimize mixtures of whey protein concentrate (WPC) and two flours of rice and maize flours for the production of gluten-free sponge cakes. This was obtained by using mixture design methodology. WPC incorporation had positive effects on specific volume and baking loss of cakes, whilst, their incorporation increased their hardness. Considering all cakes properties, two formulas F1 (78.5% Maize, 15% Rice and 6.5% WPC) and F2 (82.4% Maize, 12% Rice and 5.6% WPC) were optimized using a mixture design. The microstructure F1 was more organized and very well structured with smaller aggregates. According to the organoleptic evaluation, F1 was also most appreciated by the tasting panel. The findings of the present study indicated that maize and rice flours, and WPC could be used as a substitute for wheat flour in producing sponge cakes of high quality.

1. Introduction

Cakes are typically sweet baked dessert, consumed by almost all levels of society. This is mainly due to its ready-to-eat nature; its availability in different varieties besides its reasonable cost. Cakes are among the cereal products mainly composed of wheat flour and characterized by a flexible and elastic alveolar crumb.

Allergy to proteins in wheat flour such as coeliac disease is becoming increasingly frequent (Sicherer & Sampson, 2014). The apparent of coeliac disease or other allergic reactions/intolerances to gluten have led to develop various gluten-free products. However, the substitution of gluten presents a major technological challenge, as it is an essential structure-building protein, which is necessary for formulating high quality cereal-based products. In addition, the gluten-free foods are often more expensive than foods containing gluten, and obtaining these special foods is difficult for some patients.

On the other hand, flours from different botanical sources have been considered as partial or total substitutes for wheat flour. Hence, rice and maize are the most used cereals in these special food's elaborations. However, the replacement of wheat flour usually leads to a decrease in the quality of the products. Therefore, it is necessary to optimize new formulae to improve the qualitative characteristics of gluten-free products.

In this regards, different protein sources found applications in gluten-free products, where substitution of the structural protein complex of gluten is required in order to improve these products quality. Dairy, legumes and soybean proteins are the most used proteins in gluten-free formulations (Gularte, Gómez, & Rosell, 2012). Sahagún et al. (2018) investigated the effect of four commercial proteins sources (pea, rice, egg white and whey) on the characteristics of gluten-free layer cakes. These authors reported that whey protein cakes were among the ones with the highest acceptability by the consumers.

Whey is obtained mainly from cheese production (Díaz-Ramírez et al., 2016). Apart from being highly nutritious, whey proteins have excellent functional properties such as high solubility, foaming capability, water holding capacity and emulsifying properties (Mulvihill & Fox, 1989). Those properties are appreciated for bakery products, such as cakes, essentially when used as whey protein concentrate (WPC).

In fact, whey proteins' functionality has been described by several studies in food cakes, indicating an improvement in the dough texture (Díaz-Ramírez et al., 2016; Sahagún et al., 2018).

Aiming to improve the overall nutritional, functional and

* Corresponding authors.
E-mail addresses: imene_ammar@yahoo.fr (I. Ammar), imenef@gmail.com (I. Felfoul).

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acceptability properties of gluten-free sponge cakes, this study was conducted to optimize a novel gluten-free sponge cake formulation using rice and maize flours with the supplementation of WPC at levels of 10 and 15%. Then, the impact of this substitution on the physico-chemical, textural properties and the cost of gluten-free sponge cakes were evaluated using a mixture design methodology.

2. Material and methods

2.1. Raw materials

2.1.1. Preparation

Wheat, maize and rice grains were purchased from a local supplier (Sfax region, Tunisia). Then, the grains were ground using a mill (Model 6NFZ-2.2C, Chine) and passed through a 1 mm sieve to obtain a coarse flour, which was then packed and stored at 5 °C until use.

Fresh cow milk was purchased from a local breeding located in Sfax region (Tunisia). Once arrived at the laboratory at 4 °C, a pH determination was realized with Metrohm pH-meter. Then, milk was skimmed by centrifugation at 3000 × g during 20 min at 4 °C (Gyrozen 1580 MGR, Multi-purpose Centrifuge, Daejeon, Korea). Rennet-whey was obtained after rennet coagulation of fresh milk at 36 °C in the presence of 1.4 mL L^{-1} of microbial rennet (*M. miehei*, strength = 1:10,000; Laboratories Arrazi, Parachimic, Sfax, Tunisia). The obtained rennet-whey was freeze-dried (Thermal electron corporation, Modulyod freeze dryer, USA) in order to obtain whey protein concentrate (WPC) in fine powder (25% protein content) (Felfoul, Lopez, Gaucheron, Attia, & Ayadi, 2015).

2.1.2. Physicochemical analysis of flours

Moisture, ash, fat and protein contents were analyzed according to AOAC methods 925.10, 923.03, 920.85, 996.11 and 93 945.18B, respectively (AOAC, 1990). Protein content was calculated by multiplying azote content (N) by factor of 6.25. Starch contents of the flours were determined an enzymatic-colorimetric assay using a Megazyme Total Starch Assay Kit in accordance with AACC (method 76–13.01) (AACC, 2000). Total (TDF) and insoluble dietary fibre (IDF) expressed as g TDF or IDF/ 100 g, were determined following the enzymatic–gravimetric AOAC (method 985.29) (AOAC, 1990). Soluble dietary fibre (SDF) was calculated by subtracting the IDF proportion from TDF.

The soluble sugars were determined according to the method of de Albuquerque et al. (2020). In brief, 1 g of flour (wheat, maize or rice) was mixed with 10 mL of ultrapurified water (Milli-Q, Merck, Darmstadt, Germany) and shaken for 20 min at room temperature (25 °C), 150 rpm using an orbital shaker (Bühler, Switzerland). The mix was centrifuged (4000 × g, 15 min, 25 °C), and then the supernatant was filtered two times through qualitative filter paper and syringe filter (0.45 µm pore size, Whatman, Fisher Scientific, Schwerte, Germany). The sugar content was determined using high-performance liquid chromatography (HPLC) using an Agilent chromatograph (model 1100 Infinity LC, Agilent Technologies, St. Clara, CA, USA) equipped with refractive index detector (RID) (G1362A model).

The flour extract (20 µL) was injected onto the HPLC column (Agilent Hi-Plex 87H column) (300 × 7.8 mm) using 4 mM sulphuric acid as a mobile phase. The separation of sugars was done at a flow rate of 0.6 mL/min at 40 °C.

The identification of sugars was determined by comparison with those of the fructose, glucose and maltose standards (Sigma-Aldrich). The average peak areas were estimated assuming a linear response and that the detector response was the same for the standard and the sample using Agilent's ChemStation OpenLAB CDS software.

The particle size distribution of flours was measured using a laser diffraction particle size analyzer (Analysette 22 MicroTech plus, FRITSCH, ldar oberstein, Germany). d_{10}, d_{50}, d_{90}, and $D[4.3]$ presented the size distribution characteristics and are expressed in µm. Diameter d_x means that × % of the volume distribution is below this value. $D[4.3]$ is the equivalent volume mean diameter or the De Broncker mean diameter (Dhen, Román, Ben Rejeb, Martínez, Gargouri, & Gómez, 2016).

Swelling capacity of flours was determined according to Robertson, Monredon, Dysseler, Guillon, Amadò and Thibault (2000).

Water solubility index (WSI) of flours was determined using the method of AACC (2000). A sample of 2 g was dispersed in 100 mL of distilled water in a water bath at 80 °C during 30 min. The dispersion was then centrifuged at 1100 × g during 10 min (Gyrozen 1580 MGR, Multi-purpose Centrifuge, Daejeon, Korea) at room temperature and the supernatant was collected carefully and dried at 103 ± 2 °C to determine its solid content. WSI was expressed as the percentage of the total of the original sample that was present in the soluble fraction.

Water-holding capacity (WHC) was measured by the method of McConnell, Eastwood and Mitchell (1974). Oil-holding capacity (OHC) was measured by the method of Lin, Humbert and Sosulski (1974).

The color of samples was measured using a colorimeter (Konica Minolta, Inc, Japan) in the CIE $L^*a^*b^*$ system. The colorimetric parameters L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) were determined. The chroma (C*) and hue angle (h°), representing the saturation level and shade of the color, respectively, were calculated as follows (Saricoban & Yilmaz, 2010).

2.2. Sponge cake

2.2.1. Sponge cake preparation

The gluten-free sponge cake making was established using a mixture simplex design methodology in order to obtain all possible mixtures (15 essays) as function of the three components: two flour sources (maize: 0–100% and rice: 0–100%) and one presenting the protein enriching agent WPC (0–15%). All mixtures were carried out in triplicates. Table 1 and Fig. 1 presented the designed mixtures with the corresponding compositions.

The mathematical model applied for each response could be in linear (Eq. (1)), quadratic (Eq. (2)) or special cubic (Eq. (3)). The chosen form of the adequate model for each response was based on the fit quality using the coefficient of determination (R^2), the adjusted coefficient of determination (R^2_{Adj}) and the significant level of regression ($p < 0.05$).

$$Y = b_A \cdot A + b_B \cdot B + b_C \cdot C \quad (1)$$

$$Y = b_A \cdot A + b_B \cdot B + b_C \cdot C + b_{AB} \cdot A \cdot B + b_{AC} \cdot A \cdot C + b_{CB} \cdot C \cdot B \quad (2)$$

$$Y = b_A \cdot A + b_B \cdot B + b_C \cdot C + b_{AB} \cdot A \cdot B + b_{AC} \cdot A \cdot C + b_{CB} \cdot C \cdot B + b_{ABC} \cdot A \cdot B \cdot C \quad (3)$$

The standard formulation of sponge cake used in this study was prepared according to Levy (1981): sugar 30.1%, wheat flour 20.1%, fresh egg white 27.2%, fresh egg yolk 16.9%, full fat liquid milk of cow 4.5%, baking powder 0.6% and vanilla 0.5%. Two batters were prepared in two bowls each. In the first bowl, fresh egg yolks, 95% sugar, milk, vanilla, wheat flour and baking powder were mixed at 580 rpm for 2 min. In a second bowl, 5% sugar and fresh whites or one of the tested mixtures based on WPC powder, were mixed at 580 rpm for 3 min. Finally, the two batters were mixed together at 136 rpm for 2 min. The used mixer was using Alfawise Food Stand Mixer Dough Blender (Alfawise Stand Mixer, SM-1301Z, UK). Then, the final whole batter (120 mL) was poured into different pyrex pans (0.10 m diameter, 0.05 m height) and baked at 175 °C for 25 min in a Luxell Turbo Mini Baking Oven (Luxell Turbo Fan 3675, Turkey). After baking, the cakes were cooled at 20 °C for 120 min.

2.2.2. Physicochemical characterization of sponge cake

Moisture content and chemical composition of sponge cakes, including protein (N × 6.25), fat and sugar contents, were determined using the standard methods (AOAC, 1990), as previously described.

Cake specific volume was calculated as the ratio between the cake

Table 1
Responses to different formulations of sponge cake containing WPC, and maize and rice flours.

Mixture	Maize (A)	Rice (B)	WPC (C)	Moisture content (% DM)	Water activity	Protein content (% DM)	Energetic value (kcal/100 g)	Specific volume (cm^3/g)	WHC (g/100 g)	Baking loss (%)	Hardness (N)	Springiness	Cohesiveness	Cost (€)
Control	0	0	0	$27.92^{cd} \pm 0.29$	$0.85^{a} \pm 0.00$	$10.28^{bc} \pm 0.19$	$324^{de} \pm 1$	$2.54^{bc} \pm 0.13$	$3.06^{cd} \pm 0.42$	$14.56^{f} \pm 0.34$	$7.31^{def} \pm 0.27$	$9.20^{e} \pm 0.33$	$0.40^{a} \pm 0.01$	0.49
M1	1	0	0	$24.93^{ab} \pm 2.18$	$0.87^{d} \pm 0.00$	$12.75^{f} \pm 0.42$	$350^{f} \pm 10$	$3.81^{e} \pm 0.07$	$2.78^{bc} \pm 0.25$	$15.45^{f} \pm 0.34$	$4.53^{bc} \pm 0.55$	$11.87^{f} \pm 0.09$	$0.48^{a} \pm 0.01$	0.50
M2	0	1	0	$30.23^{ef} \pm 0.37$	$0.86^{c} \pm 0.01$	$11.25^{de} \pm 0.69$	$325^{e} \pm 2$	$5.91^{f} \pm 0.62$	$3.47^{cd} \pm 0.08$	$30.30^{j} \pm 0.42$	$4.32^{b} \pm 1.04$	$8.65^{e} \pm 1.04$	$0.40^{a} \pm 0.04$	0.50
M3	0.500	0.500	0	$28.56^{cde} \pm 0.22$	$0.86^{bc} \pm 0.01$	$9.92^{bc} \pm 0.80$	$331^{e} \pm 3$	$2.70^{c} \pm 0.13$	$3.64^{cd} \pm 0.10$	$30.84^{j} \pm 1.01$	$5.30^{bcd} \pm 0.52$	$9.64^{e} \pm 1.18$	$0.36^{a} \pm 0.02$	0.50
M4	0.250	0.750	0	$27.79^{cd} \pm 0.21$	$0.86^{bc} \pm 0.00$	$9.28^{b} \pm 0.55$	$308^{bc} \pm 0$	$2.67^{c} \pm 0.14$	$2.95^{bc} \pm 0.14$	$38.13^{h} \pm 0.98$	$7.96^{efg} \pm 0.14$	$11.58^{f} \pm 0.18$	$0.41^{ab} \pm 0.01$	0.50
M5	0.750	0.250	0	$23.48^{a} \pm 0.13$	$0.85^{ab} \pm 0.01$	$10.51^{cd} \pm 0.81$	$350^{f} \pm 2$	$2.49^{bc} \pm 0.21$	$2.88^{bc} \pm 0.21$	$28.00^{i} \pm 1.16$	$7.09^{cde} \pm 0.64$	$10.97^{f} \pm 1.08$	$0.39^{ab} \pm 0.01$	0.50
M6	0.900	0	0.100	$31.75^{f} \pm 0.66$	$0.84^{a} \pm 0.013$	$13.58^{g} \pm 1.04$	$278^{a} \pm 1$	$1.94^{a} \pm 0.03$	$2.97^{cd} \pm 0.70$	$11.70^{cd} \pm 0.31$	$9.84^{fg} \pm 0.81$	$3.82^{b} \pm 0.27$	$0.39^{cd} \pm 0.00$	0.57
M7	0	0.900	0.100	$27.62^{cd} \pm 0.07$	$0.83^{a} \pm 0.018$	$12.20^{ef} \pm 0.83$	$298^{b} \pm 1$	$3.29^{d} \pm 0.03$	$4.78^{e} \pm 0.46$	$25.39^{h} \pm 0.05$	$1.39^{a} \pm 0.11$	$0.47^{a} \pm 0.02$	$0.34^{cd} \pm 0.08$	0.57
M8	0.450	0.450	0.100	$29.11^{de} \pm 0.87$	$0.82^{ab} \pm 0.02$	$10.09^{bc} \pm 0.25$	$311^{c} \pm 4$	$2.26^{ab} \pm 0.19$	$3.04^{cd} \pm 0.05$	$12.17^{d} \pm 0.17$	$13.26^{h} \pm 2.54$	$7.13^{d} \pm 0.06$	$0.46^{d} \pm 0.01$	0.57
M9	0.225	0.675	0.100	$28.09^{cd} \pm 0.14$	$0.76^{c} \pm 0.054$	$9.20^{b} \pm 0.10$	$297^{b} \pm 0$	$4.05^{e} \pm 0.20$	$3.58^{de} \pm 0.05$	$13.26^{e} \pm 0.20$	$3.05^{ab} \pm 0.02$	$1.43^{a} \pm 0.03$	$0.47^{d} \pm 0.00$	0.57
M10	0.675	0.225	0.100	$25.70^{b} \pm 0.23$	$0.79^{bc} \pm 0.05$	$9.24^{b} \pm 0.03$	$331^{e} \pm 8$	$2.02^{a} \pm 0.04$	$1.26^{a} \pm 0.06$	$10.20^{a} \pm 0.07$	$8.79^{efg} \pm 2.88$	$3.58^{b} \pm 1.22$	$0.41^{cd} \pm 0.01$	0.57
M11	0.850	0	0.150	$29.96^{def} \pm 1.26$	$0.80^{a} \pm 0.023$	$8.99^{b} \pm 0.54$	$312^{c} \pm 15$	$1.94^{a} \pm 0.09$	$1.19^{a} \pm 0.12$	$10.64^{bc} \pm 0.07$	$9.85^{fg} \pm 0.85$	$3.59^{b} \pm 0.03$	$0.37^{cd} \pm 0.03$	0.60
M12	0	0.850	0.150	$26.72^{bc} \pm 2.46$	$0.80^{a} \pm 0.018$	$7.15^{a} \pm 0.39$	$305^{bc} \pm 12$	$1.93^{a} \pm 0.05$	$1.81^{ab} \pm 0.38$	$18.09^{g} \pm 0.12$	$3.61^{ab} \pm 1.12$	$0.96^{a} \pm 0.40$	$0.26^{bc} \pm 0.03$	0.60
M13	0.425	0.425	0.150	$29.74^{def} \pm 0.22$	$0.79^{ab} \pm 0.00$	$7.20^{a} \pm 0.26$	$296^{b} \pm 4$	$2.23^{a} \pm 0.07$	$2.54^{bc} \pm 0.06$	$9.54^{a} \pm 0.04$	$10.28^{g} \pm 2.59$	$4.38^{b} \pm 1.06$	$0.43^{d} \pm 0.00$	0.60
M14	0.2125	0.6375	0.150	$30.60^{ef} \pm 2.14$	$0.77^{c} \pm 0.011$	$7.63^{a} \pm 0.59$	$313^{cd} \pm 11$	$1.96^{a} \pm 0.11$	$2.39^{bc} \pm 0.12$	$10.54^{ab} \pm 0.31$	$16.92^{i} \pm 1.74$	$6.58^{cd} \pm 0.77$	$0.39^{cd} \pm 0.01$	0.60
M15	0.6375	0.2125	0.150	$30.22^{def} \pm 0.91$	$0.75^{bc} \pm 0.06$	$10.13^{bc} \pm 0.24$	$328^{e} \pm 6$	$2.03^{a} \pm 0.14$	$2.38^{bc} \pm 0.33$	$10.23^{a} \pm 0.03$	$14.11^{h} \pm 1.72$	$5.78^{c} \pm 0.72$	$0.41^{cd} \pm 0.00$	0.60

Means with different superscript letters in the same column are significantly different according to Duncan's test ($p < 0.05$).

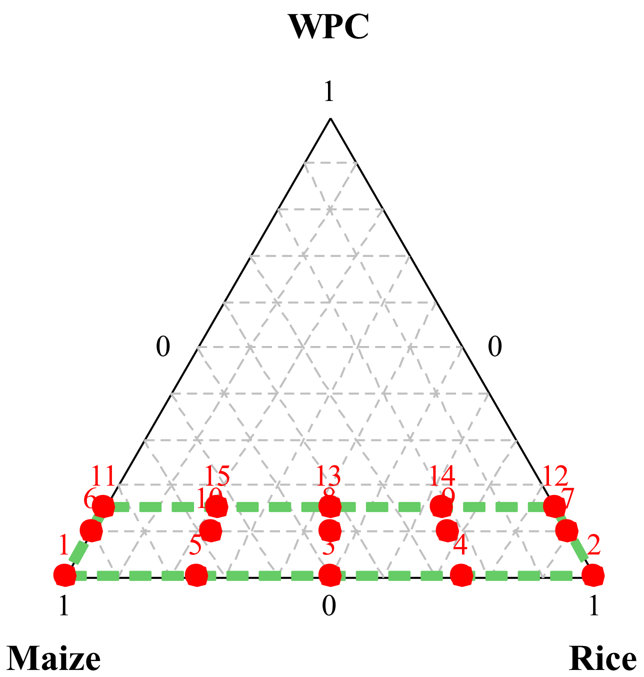

Fig. 1. Simplex design plot in proportions

volume and its weight (Lee, Hoseney, & Varriano-Marston, 1982) with a volume accuracy of ± 10 cm^3.

The baking loss (%) was determined using the Eq. (4). Cake baking loss and specific volume were assessed on six independent cake samples from each type, 24 h after baking.

$$\%\boldsymbol{Bakingloss} = \frac{(\boldsymbol{W_f} - \boldsymbol{W_0})}{\boldsymbol{W_0}} \times 100 \quad (4)$$

2.2.3. Texture analysis of sponge cake

Texture of cake samples was determined using Textural Profile Analysis (TPA) test. A Texture Analyser (LLOYD instruments, Fareham, England) was used to measure the force–time curve for two cycles of compression (Mallek et al., 2012). All measurements were carried out in controlled room at 25 °C. The measurements were carried out on 60 mm-width × 60 mm-length × 40 mm-height cake samples. An aluminum cylinder probe was used to compress the cake to 50% (20 mm) of its original height with a displacement speed of 2 mm/s. As recommended by Gómez, Ruiz and Oliete (2011), the upper dome and crust sides were removed from all cake samples. Texture profile parameters, hardness (N), cohesiveness and springiness were measured. Texture analysis was assessed on three independent cake samples from each type, 24 h after baking.

2.2.4. Cake microstructure

Sponge cake optimized formulations were mounted on carbon sample holders using double-side sticky tape and observed using a JEOL JSM-5400 scanning electron microscope JSM-7100F to Field Emission (JEOL Ltd, Tokyo Japan) with the LM mode at 15 kV accelerating voltage. Samples were sputtered with 20 nm of gold using a JEOL JFC-1100E ion sputtering device (Fine Coat). Micrographs at 50 ×, 500 × and 2000 × magnification are presented.

2.2.5. Sensory evaluation

A sensory evaluation of sponge cakes was conducted as previously described by Ayadi, Abdelmaksoud, Ennouri and Attia (2009). The sensory test of the cake samples was conducted after 24 h of baking at 20 °C. The panel was composed of 60 volunteers (21–50 years of age), which were previously trained about the meaning of the sensory attributes and scores.

Each of the cake samples studied in this paper was hermetically sealed with a plastic film (Plastic Wrap Food Wrap Cling Film, China) and coded with three-digit random numbers, and randomly presented to the panel (Díaz-Ramírez et al., 2016). After coding the samples, panelists were instructed to rate appearance, odor, taste, texture, and overall impression by using a 6 – point hedonic scale: with 0 = dislike extremely and 5 = like extremely. The control cake was presented simultaneously with the rest of samples. The panelists rinsed their mouth with water between samples. All samples were analyzed one day after baking.

2.3. Statistical analysis

The entirety of the executed experiments was conducted in triplicate and differences between treatment means were determined via the Duncan's procedure at $p < 0.05$ using the SPSS statistics 19. The design, the mathematical modelling and all statistical tests ($p < 0.05$) of the experimental mixture design were carried out using Minitab 16 (Minitab Inc, Launcher).

3. Results and discussion

3.1. Physicochemical characterization of flours

The proximate composition, functional properties and color parameters of wheat (control), maize and rice flours are summarized in

Table 2
Physico-chemical characteristics of flour samples.

	Wheat	Maize	Rice
Proximate composition			
Moisture (%)	$7.57^a \pm 0.05$	$9.64^b \pm 0.06$	$9.73^b \pm 0.36$
Proteins (g/100 g DM)	$9.31^a \pm 0.86$	$7.31^b \pm 0.27$	$5.97^c \pm 0.43$
Ash (g/100 g DM)	$1.60^a \pm 0.22$	$1.13^b \pm 0.29$	$0.35^c \pm 0.22$
Fat (g/100 g DM)	$1.52^a \pm 0.06$	$5.19^b \pm 0.27$	$1.47^a \pm 0.05$
Starch (g/100 g DM)	$80.00^b \pm 4.03$	$76.72^c \pm 3.62$	$82.48^a \pm 4.32$
SDF (g/100 g DM)	$1.5^a \pm 0.3$	$0.31^c \pm 0.09$	$0.51^b \pm 0.05$
IDF (g/100 g DM)	$2.7^a \pm 0.1$	$1.95^b \pm 0.02$	$1.11^c \pm 0.06$
TDF (g/100 g DM)	$4.2^a \pm 0.6$	$2.26^b \pm 0.8$	$1.62^c \pm 0.09$
Sugars (g/L)			
Fructose	$0.50^a \pm 0.1$	$0.61^b \pm 0.05$	NF
Glucose	$0.93^a \pm 0.2$	$1.17^b \pm 0.01$	$1.28^b \pm 0.07$
Maltose	$4.02^a \pm 0.5$	$4.76^a \pm 0.20$	$1.49^b \pm 0.30$
Particle size characteristics			
d_{10} (μm)	$57.80^c \pm 2.69$	$86.05^b \pm 0.29$	$108.00^a \pm 12.45$
d_{50} (μm)	$574.20^b \pm 1.98$	$599.95^a \pm 2.05$	$467.55^c \pm 20.72$
d_{90} (μm)	$878.60^b \pm 25.6$	$952.75^a \pm 5.16$	$805.30^c \pm 28.43$
$D[4.3]$ (μm)	$511.35^b \pm 39.39$	$567.35^a \pm 4.31$	$466.30^c \pm 19.09$
Functional properties			
Swelling capacity (cm^3/g)	$5^a \pm 0.00$	$5^a \pm 0.00$	$5^a \pm 0.00$
Water solubility index (%)	$14.97^a \pm 0.57$	$14.75^a \pm 0.72$	$13.45^b \pm 0.12$
Water-holding capacity (g/100 g)	$2.62^a \pm 0.28$	$2.79^a \pm 0.40$	$2.84^a \pm 0.17$
Oil-holding capacity (g/g)	$4.18^a \pm 0.92$	$8.74^b \pm 1.89$	$5.35^a \pm 1.38$
Color parameters			
L*	$74.21^b \pm 0.01$	$71.4^c \pm 0.01$	$88.28^a \pm 0.01$
a*	$3.56^b \pm 0.26$	$6.75^a \pm 0.01$	$0.20^c \pm 0.00$
b*	$19.93^b \pm 0.00$	$36.85^a \pm 0.01$	$6.30^c \pm 0.01$
C*	$20.24^b \pm 0.05$	$37.46^a \pm 0.01$	$6.30^c \pm 0.00$
h°	$79.53^a \pm 0.20$	$79.62^a \pm 0.01$	$91.83^b \pm 0.01$

Means with different superscript letters in the same row are significantly different according to Duncan's test ($p < 0.05$).
DM = Dry Matter; TDF = Total dietary fibre; SDF = Soluble dietary fibre; IDF = Insoluble dietary fibre; NF = Not Found.

Table 2. Moisture contents of wheat, maize and rice flours were 7.57, 9.64 and 9.73%, respectively, with no significant difference between maize and rice flours. Wheat flour has significantly the highest ash content value compared to maize (1.13%) and rice (0.35%) flours. Fat content of maize flour (5.19%) is significantly higher than those of wheat and rice flours (1.52 and 1.47%, respectively). Protein contents of wheat, maize and rice flours were 9.31, 7.31 and 5.97%, respectively (Table 2). The results were similar to those of Clerici, Arioldi and El-Dash (2009) and Torbica, Hadnadev and Hadnadev (2012). These authors reported that wheat flour was characterized by higher protein, ash and fat contents than rice flour. About starch content, the value found for rice flour was the highest.

The total, insoluble and soluble dietary fiber (TDF, IDF and SDF, respectively) contents of the flours are shown in Table 2. Variable contents of total dietary fiber were observed among the tested flours. The highest contents of TDF were found in wheat flour. In fact, it is known in the literature that gluten-free flours (maize and rice) have low levels of fiber (Hosseini, Soltanizadeh, Mirmoghtadaee, Banavand, Mirmoghtadaie, & Aliabadi, 2018). The fiber contents were characterized by higher IDF content for all the tested flours.

Fructose, glucose and maltose were found in variable contents. The content of maltose was the highest among the tested sugars with higher content for wheat and maize flours (4.02 and 4.76 g/L respectively) than rice flour (1.49 g/L). Fructose was not found in the rice flour. In general, maize and wheat flours tend to provide higher nutrients than rice flour (Table 2), which is consistent with the results obtained by Kraithong, Lee and Rawdkuen (2018).

Particle size distribution of wheat, maize and rice flours was also investigated in Table 2. Indeed, the studied three types of flours consisted mainly of average particle diameter ranging from 467.55 µm for rice flour to 600 µm for maize flour. The observed variation could be related to different factors such as the difference in friability of the different seeds or may be to technological factors during the grinding operation. Flour color is of importance because it affects directly the color of the cake crumb. In fact, wheat, maize and rice flours significantly varied in color (Table 2). Rice flour had significantly the highest L* value (88.28), which means that it was the whitest one. Maize flour, on the other hand, had the lowest L* value (71.4), which means that it was the darkest flour. Moreover, rice flour had the highest h° value that was significantly different from the other flours. Overall, rice flour is the brightest due to its higher L* and h° and lower a* and b* values.

There are no significant differences in water absorption and swelling capacities among all the flours (Table 2). This shows that the studied flours have the same baking quality since this criterion is function of the WHC (Shittu, Dixon, Awonorin, Sanni, & Maziyadixon, 2008).

On the other hand, the OHC of the flours ranged from 4.18 to 8.74 g of oil/g of flour (Table 2). Maize flour had the highest OHC value while wheat flour had the lowest one. Crude fat content correlates with functional properties. In fact, high flour lipid content significantly decreases cake volume (Guine & Correia, 2014).

3.2. Physicochemical characterization of sponge cake

The proximate composition, physicochemical characteristics and textural parameters of the different gluten-free sponge cake formulations tested with the control sample are shown in Table 1. No significant difference was found in cakes moisture contents between the mixtures studied. This means that the flour combinations as well as WPC did not have any clear effect on cakes moisture content. However, the corresponding water activity (a_w) shows variations between different mixtures. In fact, the formulated gluten-free sponge cakes without addition of WPC have water activity values higher than 0.85. Food product with water activity value more than 0.85 can be considered as moist food product (Smith and Simpson, 1995). Thus, gluten-free sponge cakes without WPC fell into the moist food category and they are susceptible to microbial spoilage. However, gluten-free sponge cakes prepared with WPC showed significantly lower water activity than that of control sample (0.85) (Table 1). The addition of 10% WPC significantly decreases the water activity to reach 0.76 for M9 and 0.79 for M10 as shown in Table 1. The water activity decrease was more significant with 15% of WPC addition. Shevkani & Singh (2014) also reported that the addition of different proteins isolates decreases water activity in the gluten-free muffin formulations. The decrease in water activity of cakes prepared with WPC incorporation might be attributed to water holding capacity of WPC. This show that WPC could plays an important role in extended storage life of cakes.

Protein contents of gluten-free sponge cakes without WPC addition ranged between 9.28 and 12.75% (Table 1). These samples differed significantly from control sample. Otherwise, the more the percentage of maize flour used was, the higher the protein content of gluten-free sponge cake was. This could be due to the higher protein content in maize flour compared to rice flour (Table 2). Recently, it has been suggested that protein content was higher for finest particles but lower for intermediate and coarser particles (Dhen et al., 2016). According to Table 3, the interaction of only maize or rice flour with WPC had significantly positive and very high coefficient, indicating increased protein content. However, protein content decreased when using the maize and rice flours' mixture with WPC, mainly with raising the level of WPC (15%) (Table 1). In general, in the preparation of cakes, flour is not the only ingredient that provides protein. Eggs also represent 30 to 50% of the total protein content of cakes (Dewaest et al., 2017). It has been reported that the cell walls of cakes are made of a protein network combination consisting of egg proteins and gluten (Wilderjans et al., 2010; Dewaest et al., 2017). It is clear from Table 1 that the addition of 15% WPC had a negative effect on egg proteins during the preparation of cakes. Table 1 shows also water holding capacity (WHC), baking loss percentage (W) and specific volume variation between the different formulations of sponge cakes. WHC values of samples without WPC addition are not significantly different from the control sample. The differences are shown in mixtures with 15% WPC incorporation which is due essentially to the higher concentration of WPC added. Early study has shown that water absorption depends on two main factors: protein content and starch content (Tipples, Kilborn, & Preston, 1994). Indeed, the starch content is higher for the used rice and wheat flours than for maize flour (Table 2). The addition of WPC led to decreased WHC values. Because of their amphiphilic nature, proteins have a certain affinity for a water–air interface. After adsorption, proteins tend to interact and form a viscoelastic film at the interface (Wouters Arno et al., 2017).

On the other hand, in the absence of WPC, the cakes M3-M5 had significantly similar specific volume than the control with highest value in samples containing 100% of maize or rice flour (M1 and M2, respectively). Whey protein exhibited an opposite behavior, as 10% of WPC increased the specific volume while 15% reduced it significantly (Table 1). This observation may be related to the reverse effect of increased amount of protein. This corroborates with the result of Díaz-Ramírez et al. (2016) where 50% and 100% substitution samples with whey protein isolate presented significantly lower specific volume values.Moreover, cake volume was not only significantly affected by the WPC concentration, but also by the flour source, indicating that WPC have different effect depending on the flour's source used in the formula. As seen in Table 1, samples containing higher percentage of rice flour (M2, M7 and M9) provided the highest specific volume. According to Table 3, interaction of maize and rice flours with WPC had different positive coefficients, indicating increased specific volume differently. The possible reason for the observed improvements in specific volume in the presence of maize and rice flours with WPC might be attributed to the more air incorporated during the fermentation or baking process, resulting in a cake with high specific volume (Zhou, Faubion, & Walker, 2011). Baking loss percentage is a crucial parameter for the structural transformation of the cake. The gas escaping during the baking step could explain this loss. It can be noted that the baking loss rate decreases

Table 3
Regression results of different studied responses as function of three components.

Response	Coefficients							Statistics		
	A (Maize)	B (Rice)	C (WPC)	AB	AC	BC	ABC	p	R^2 (%)	R^2_{Adj} (%)
Moisture content (%)	24.79**	30.29**	78.19**	−4.36	−14.42	−78.65	47.09	<0.001	49.68	41.74
Protein content (% DM)	12.9***	11.4***	−309.8***	−11.6***	356.0***	350.4***	24.7	<0.001	73.48	69.30
Energetic value (kcal)	351**	319**	2853**	−17	−3305**	−3104**	627	<0.001	54.12	46.88
Specific volume (g/cm^3)	3.7***	5.7***	−31.6***	−9.8***	24.0	15.2	82.2***	<0.001	81.85	78.99
WHC (g/100 g)	2.8**	3.7**	−123.0**	−0.7	136.9**	141.0**	8.8	<0.001	49.80	41.87
Baking loss (%)	15.6***	32.2***	348.2***	37.1***	−419.0**	−470.6**	−544.6***	<0.001	95.08	93.23
Hardness (N)	5.3	4.3	394.8	8.2	−413.0*	−458.7*	182.9*	<0.001	59.84	53.50
Springiness	11.7***	9.1***	502.1***	1.2	−639.8***	−641.8***	126.0**	<0.001	87.13	85.10
Cohesiveness (–)	0.461*	0.415*	−4.422*	−0.250**	4.781*	4.580*	6.453***	<0.001	70.00	65.27
Springiness	11.7***	9.1***	502.1***	1.2	−639.8***	−641.8***	126.0**	<0.001	87.13	85.10
Cost (€)	0.5***	0.5***	1.17***					<0.001	99.86	99.86

p: p-value (dimensionless); R^2: coefficient of determination (%); R^2_{Adj}: Adjusted coefficient of determination (%).
*: significant effect ($p < 0.05$); **: very significant effect ($p < 0.01$); ***: very highly significant effect ($p < 0.001$).

by the presence of WPC and the effect was more pronounced at 15% especially. Table 1 summarizes also the textural properties of the sponge cake formulations. Interestingly, before WPC addition, hardness values decreased when maize and/or rice flour was only used. According to Table 1, mixtures with high level of rice flour (M2, M7, M9 and M12) were able to retain gas-yielding cakes with higher volumes and lower hardness values. The sample prepared with the two flours combination was harder than the others. Experimental results obtained for hardness showed that the interaction between rice or maize flour with WPC had negative coefficients, indicating decreased hardness values (Table 3). Samples containing blend of three components, hardness values increased significantly. Similarly, increased hardness value has been reported in other studies (Sahagún, Bravo-Núñez, Báscones, & Gómez, 2018), in which the lower the specific volume was, the higher the cake hardness was.

Hardness values increased with the addition of WPC (Tables 1 and 3). For instance, hardness changed from 7.31 N for control sample to 16.92 N for M14. According to Díaz-Ramírez et al. (2016), the increment of hardness of whey protein could be due to its high solubility and then the available water for sugar solubility was reduced which cause its crystallization when exposed to heat. Besides, at higher temperature, the solubility of whey proteins is lost due to their heat sensitivity. This heat denaturation is mainly due to the rupture of the disulphide bridges within the molecule which causes its denaturation (De Castro et al., 2017). In our study, a temperature of 175 °C was used for baking the cake samples, which is sufficient for their denaturation and subsequent aggregation, thus forming a solid protein network (Felfoul et al., 2015). This would modify the cake texture and increase its hardness.

The addition of WPC also reduced the springiness and the cohesiveness (Tables 1), with greater effect seen with 15% of WPC. According to Wilderjans et al. (2010), protein aggregation is associated with springiness. Additional research works are required to confirm that WPC incorporation can stimulate protein aggregation as possible reasons for the increased hardness and reduced springiness of the cakes.

Table 1 shows the increasing production costs of gluten-free sponge cakes comparing to control cake. This increase is about 1% without the use of WPC, 8 and 11% with the enrichment of 10 and 15% of WPC, respectively. Apart from the formulation approach, operating costs need to be taken care in gluten-free product. WPC incorporation no doubt improves the gluten-free formulations, but also incurs costs due to ingredient addition. Further insights could minimize the extra costs for gluten-free sponge cakes.

3.3. Optimization of the sponge cake quality

Table 1 presents the studied responses results for all designed mixtures previously. All results present very low standard deviations, displaying the repeatability of all obtained results in term of responses for all studied mixtures. Table 3 presents models coefficients (Eqs. (1), 2 and 3) for each response. The statistical parameters showing fitting quality are also presented for each response. All the obtained results in paragraph 3.2 were confirmed by the adopted modelling protocol of mixture design ($p < 0.05$).

The fitting quality of the different regressions are considered as interesting basing on: significant obtained regressions for all responses ($p < 0.001$); very interesting coefficients of determination ($50 \le R^2 \le 100\%$); very important adjusted coefficients of determination ($50 \le R^2_{Adj} \le 100\%$). All the tested responses (Tables 1 and 3) and their established regressions were used to determine the two better compositions (F1 and F2, Table 4) of cake in order to obtain the better optimized cake quality. In fact, the first formulation (F1) was obtained when the degree of importance between responses has been considered in the optimization protocol. i. e. the higher degree of importance was attributed to the cost and then the second was attributed to the energetic value. The second formulation (F2) was determined without considering this criterion. The optimization of the different formulations was carried out in order to get a formulation as closed as possible to the values obtained for the control sample. Formulations were optimized by Minitab software.

Table 4
Color and sensorial analysis of optimized sponge cake.

(A)				
(%)		F1	F2	
Maize		78.5	82.4	
Rice		15	12	
WPC		6.5	5.6	
Total		100	100	
(B)				
		Control	F1	F2
Crust	L*	$46.37^{b} \pm 0.01$	$44.63^{c} \pm 0.01$	$54.92^{a} \pm 0.08$
	a*	$16.24^{a} \pm 0.01$	$13.52^{b} \pm 0.01$	$6.26^{c} \pm 0.01$
	b*	$29.01^{a} \pm 0.04$	$26.64^{a} \pm 0.04$	$31.79^{a} \pm 0.01$
	C*	$33.25^{a} \pm 0.04$	$24.87^{b} \pm 7.12$	$32.40^{a} \pm 0.01$
	h°	$60.76^{c} \pm 0.01$	$63.1^{b} \pm 0.01$	$78.87^{a} \pm 0.01$
Crumb	L*	$58.36^{a} \pm 0.01$	$63.46^{b} \pm 0.40$	$58.43^{a} \pm 0.04$
	a*	$3.24^{c} \pm 0.01$	$4.33^{b} \pm 0.13$	$5.85^{a} \pm 0.01$
	b*	$25.63^{c} \pm 0.01$	$31.27^{b} \pm 0.16$	$38.46^{a} \pm 0.01$
	C*	$25.83^{c} \pm 0.01$	$31.75^{b} \pm 0.17$	$38.90^{a} \pm 0.00$
	h°	$82.81^{a} \pm 0.02$	$82.12^{a} \pm 0.20$	$81.35^{a} \pm 0.01$
(C)				
		Control	F1	F2
Appearance		$3.73^{a} \pm 0.93$	$4.15^{b} \pm 0.93$	$3.59^{a} \pm 1.07$
Flavor		$3.27^{b} \pm 1.35$	$3.32^{a} \pm 1.16$	$2.89^{c} \pm 1.37$
Odor		$3.96^{a} \pm 0.84$	$3.84^{b} \pm 1.12$	$3.54^{c} \pm 1.10$
Texture		$3.76^{a} \pm 1.26$	$3.68^{b} \pm 1.11$	$3.50^{c} \pm 1.14$
Overall impression		$3.88^{b} \pm 0.74$	$3.81^{b} \pm 0.91$	$3.34^{a} \pm 1.01$

Means with different superscript letters in the same row are significantly different according to Duncan's test ($p < 0.05$).

3.4. Analysis of the optimum formulation gluten-free cake

3.4.1. Microstructure of optimized sponge cake

Fig. 2 presents the SEM images of gluten-free sponge cakes obtained from the optimized formulations F1 and F2 in comparison with control sample. These SEM images were taken at the same magnifications (×50; × 500 and × 2000).

The gluten-free sponge cake corresponding to control formulation (Fig. 2(a)) exhibited an irregular distribution of solids corresponding to starch granules. The components of batter formulations consisting of lipids, proteins and amylose/amylopectine were overlapping with the observed starch granules. These components could interact to form many complexes, such as lipid-protein and amylose-lipids (Gerits, Pareyt, & Delcour, 2014). The incorporation of WPC generated a granular-like microstructure where the starch granules with 50-μm thickness can be easily observed (F1 (e)). The microstructure of the F1 sponge cake formulation containing 6.5% of WPC content was more organized and very well structured with smaller aggregates unlike F2 sponge cake formulation with 5.6% of WPC content (Fig. 2 (e and f)). Fig. 2(i) reveals that F2 sponge cake formulation shows a more amorphous structure than that of F1 formulation (Fig. 2(h)).

These results indicated that the difference in structure could be due to the difference in the composition between both batters of F1 and F2 formulations. A higher content of WPC (6.5%) could contribute to a more compact sponge cake structure, which is the case of F1 sponge cake in this study. On the other hand, the average particle diameter of rice

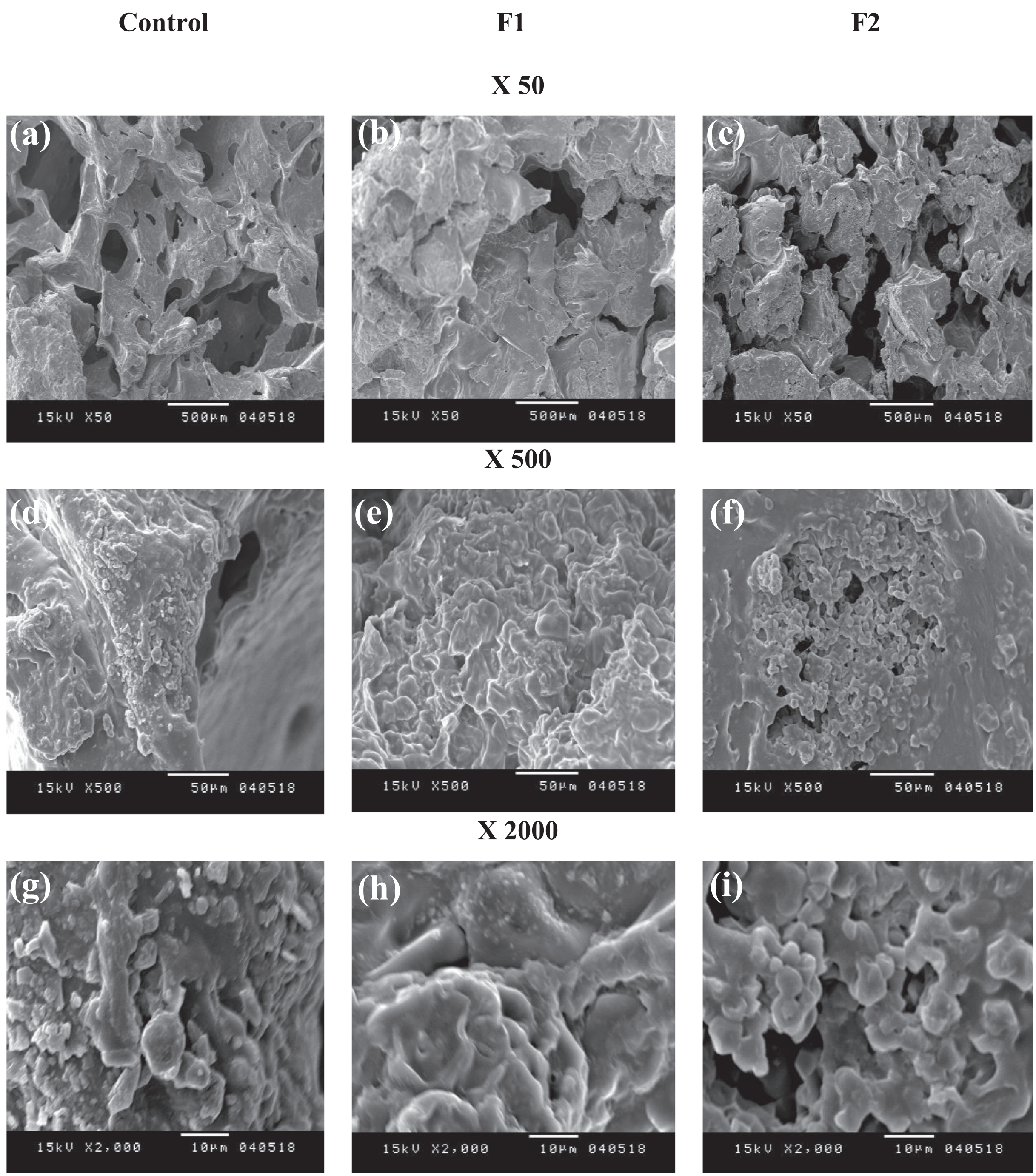

Fig. 2. Scanning electron micrographs of the optimized sponge cake formulations. Scale bars are given in each case.

flour was observed to be lower than that of maize flour (466 μm *vs* 567 μm), as shown in Table 2. As can be seen in Fig. 2, F1 (h) has a more compact structure than F2 (i). This could be due to higher concentration of rice flour in the F1 batter formulation in comparison with F2 formulation. These results are in accordance with those of Mancebo et al. (2016) who have demonstrated that fine-grained flours, could contribute to more compact dough.

Moreover, it was observed from Fig. 2 that the shape of the formed aggregates in the case of F1 formulation (Fig. 2(h)) is more circular than that of the control sample (Fig. 2(g)). Material science has established that as the pore shape gets more circular, its thermal resistance to heat transfer is lower (Polaki, Xasapis, Fasseas, Yanniotis, & Mandala, 2010). Whey proteins have been reported to be very heat sensible (Singh & Havea, 2003). Based on these findings, this result could be explained by the heat-denaturation of the protein during baking which changes the protein's elasticity and its interactions with the surrounding medium (mainly starch) (Díaz-Ramírez et al., 2016). Starch granules of rice flour are very small in diameter and have a narrow size distribution (Vallons, Ryan, & Arendt, 2011). Starch granules of maize flour were larger than those of rice flour (Table 2). The interaction of starches from rice and maize flours with WPC will lead to larger agglomerated granules as observed in Fig. 2 (i). In contrast to that, smaller spherical granules were observed for starch from wheat flour after baking (Fig. 2(g)). These findings were in accordance with those reported by Naruenartwongsakul, Chinnan, Bhumiratana and Yoovidhya (2008).

3.4.2. Color analysis of optimized sponge cakes

Table 4 presents the color parameters of the sponge cake formulations. The evaluation of both the crust and the crumb was studied of each optimized formulation. In the case of the crust, it is clear from Table 4 that h° value increased significantly from 60.76° for the control sample to 63.10° and 78.87° for F1 and F2, respectively. This variation of h° resulted in an increase of b* value and a decrease of a* value (Table 4). This indicates that the color of the sponge cake samples tends to be yellowish. This was accompanied with a significant increase in brightness L*, from 46.37 for the control sample to 54.92 for the sponge cake F2, and a decrease in brightness C*, 33.25 for the control sample to 24.87 and 32.40 for F1 and F2, respectively. The observed variations of the color attributes (L *, C * and h°) are significant for all the samples.

For the crumb color, the brightness C* is more prominent for the F1 formulation. Thus, the crumb of the F1 formulation tends to be yellow. C* is clearer for F2 than for the other samples. In fact, the color of the crumb is related not only to the color of the flour used, but also to the Maillard reactions that occur during the baking process (Aremu, Olaofe, & Akintayo, 2007). Moreover, Mancebo et al. (2016) have shown that cakes made from maize flour are more yellowish because of their higher carotenoid content, which is consistent with the obtained results of our study.

3.4.3. Sensorial analysis of optimized sponge cakes

The results of sensory analysis of the gluten-free sponge cake are presented in Table 4 (C). Panelists evaluated these cake samples according to appearance, taste, odor, texture and overall acceptability using grades from 0 to 5. These samples were served at room temperature to 60 panelists homogeneously, with mineral water to neutralize the taste receptors between the different samples.

As shown in Table 4, sponge cake made from the F2 showed the lowest score in the tested properties. While the scores for appearance, flavor and overall impression were significantly highest for F1.

It is clear from Table 4 that the control sample was most appreciated for odor and texture attributes by the tasting panelists with average scores of 3.96 and 3.76, respectively. Odor and texture scores showed significant differences between F1 and F2 formulations compared to control sample. Besides, the difference between control and F1 sponge cake formulation was not significant for the overall appreciation. Therefore, it can be concluded that F1 formulation is the most appreciated by the tasting panelists along with the control sample (Table 4).

4. Conclusion

The present study describes the optimization of a novel formulation of gluten-free sponge cake using various combinations of maize and rice flours, and additional enrichment with whey proteins (WPC). The results of this study indicated that it is possible to obtain gluten-free sponge cakes with a similar quality to sponge cakes made with wheat flour, without any additives. Nevertheless, the incorporation of WPC had positive effects on the gluten-free sponge cakes mainly at 10% level. Generally, WPC incorporation produced highest cake volume with a decrease in baking loss, but increased hardness. Two formulas F1 (78.5% Maize, 15% Rice and 6.5% WPC) and F2 (82.4% Maize, 12% Rice and 5.6% WPC) were optimized to achieve gluten-free sponge cakes with the adequate characteristics. The analysis of these optimum formulations shows that the microstructure of the F1 sponge cake formulation containing 6.5% of WPC content was more organized and very well structured than F2. The cakes of each optimized formulation were significantly different in color evaluation of both the crust and the crumb from control cakes. According to organoleptic evaluation, the optimum formulation substitution of 6.5% of the flour by WPC was most appreciated by the tasting panel. This is the first time to study the effect of the enrichment of WPC in gluten-free sponge cakes in the literature. Accordingly, a successful and novel formulation of gluten-free sponge cakes substituted with rice and maize flours, and WPC was developed. Future works should determine the quality of the obtained cakes by measuring their rheological characteristics.

CRediT authorship contribution statement

Imène Ammar: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Houda Gharsallah:** Methodology. **Abir Ben Brahim:** Methodology, Formal analysis, Investigation. **Hamadi Attia:** Conceptualization, Supervision. **M.A. Ayadi:** Conceptualization, Supervision. **Bilel Hadrich:** Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Imène Felfoul:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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