

# Gluten-Free Bread Enriched with Vegetable Flours

Maria A. Saccotelli, Sara Spinelli, Amalia Conte\*, Matteo A. Del Nobile

Department of Agricultural Sciences, Food and Environment, University of Foggia, Foggia, Italy

Email: \*amalia.conte@unifg.it

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## Abstract

The effects of different vegetable flours (broccoli, cauliflower, artichoke, fennel, zucchini and mushroom) added to gluten-free bread on sensory quality, antioxidant properties and glycemic response were assessed. Among the vegetable flours, the addition of fennel flour significantly improved sensory bread quality. Artichoke flour has the highest phenolic ( $26.51 \pm 1.92$  mg/g dw) and flavonoid content ( $26.43 \pm 1.93$  mg/g dw). Even though the content of total phenol and flavonoids in vegetable flours was higher when compared to supplemented bread, the addition of artichoke and zucchini flours increased the total phenolic and flavonoid content and improved antioxidant activity. The incorporation of high level of vegetable flours (15%) also decreased the glycemic index of bread, in particular with artichoke and zucchini flours ( $59 \pm 1.21$  and  $62 \pm 0.49$ , respectively). To sum up, the results are very interesting because the addition of vegetable flours into gluten-free bread can improve nutritional and sensory properties of bread.

## Keywords

Gluten-Free Bread, Vegetable Flours, Antioxidant Activity, Glycemic Response

## 1. Introduction

Nowadays, there is a growing interest in gluten-free products as celiac disease is increasing. Many commercially available gluten-free breads are of lower quality than their counterparts containing gluten [1]. The formulation of gluten-free bakery products presents a major challenge for the food industry. The main defects of gluten-free bread consist in a low quality, unstable structure, friable and dry crumb, lack of aroma and mouth feel [2]. In numerous studies, various technological parameters and formulations were studied to improve the quality

of gluten-free bread using starches [1], dairy products, gums and hydrocolloids [3]. Rice flour is one of the most suitable cereal flour for gluten-free products because it has a low level of prolamine and possesses unique nutritional, hypoallergenic, colorless, and bland taste properties [4]. Gluten-free bread is also poor from a nutritional point of view. The addition of compounds from fruit and vegetables are considered good ingredients to increase healthy properties [5] [6]. Recent studies have shown that herbal fortification of white bread could be considered a new trend to improve its nutritional value [7], being vegetable products rich in bioactive compounds (carotenoids, vitamins, phenolic compounds) and dietary fibers. No studies have been found on gluten-free bread enriched with vegetables. Therefore, the objective of this study was to determine the effect of different forms of fortification by using various vegetable flours. Both sensory and chemical properties were investigated to assess the product quality.

## 2. Materials and Methods

### 2.1. Raw Materials and Chemicals

Commercial corn-starch was obtained from Farmalabor s.r.l. (Canosa di Puglia, Italy), rice flour was purchased from Agostini Mill (Montefiore dell'Aso, Italy), potato starch, compressed fresh yeast, sugar, salt and sunflower oil were bought from local market. The whey protein isolate (WPI) was provided from Perrin's Chemicals s.a.s. (Bari, Italy), baking soda, cream of tartar and guar seed flour were supplied from Farmalabor s.r.l. (Canosa di Puglia, Italy).

The investigated vegetables, broccoli, cauliflower, artichoke, fennel and zucchini were provided by Farris farm (Troia, Foggia, Italy), mushroom were purchased from Hermes s.n.c. (Noicàtaro, Bari, Italy). The samples were dried at 35°C in a dryer (SG600, Namad, Roma, Italy) for 48 hours. The dried vegetable was reduced in a fine powder by a hammer mill (16/BV-Beccaria s.r.l., Cuneo, Italy) and then was sieved with a Retsch Vibratory Sieve Shaker AS 300 (Haan, Germany) to obtain a median particle size less than 63 µm.

All chemical reagents (analytical grade) were supplied from Sigma-Aldrich (Milan, Italy).

### 2.2. Bread-Making Process

The reference bread sample (named as B\_CTRL) was prepared using the following ingredients: corn starch (330 g), rice flour (300 g), potato starch (300 g), compressed fresh yeast (36 g, previously dissolved in warm water), sugar (60 g), salt (18 g), sunflower oil (18 g), whey protein isolate (WPI) (60 g), baking soda (18 g), cream of tartar (36 g), guar seed flour (12 g) and water (840 g). The ingredients were first mixed using a mixer (Conti Impastatrici, Verona, Italy) at low speed (110 rpm) for 5 min, and then at a higher speed (200 rpm) for further 10 min. Thus, the obtained dough was placed in the incubator (Thermogel, Varese, Italy) at constant temperature (30°C) and RH (85%). After 90 min, the dough was divided into pieces of 670 g, shaped in apposite moulds and placed in

incubator for further 60 min. Finally, after second proofing, the samples were baked in a pre-heated electric oven (Europa Forni, Vicenza, Italy) at 230°C for 10 min, followed by 40 min at 200°C. Baking process was performed in triplicate. After baking, breads were cooled to room temperature for 3 h and sliced 15 mm thick, removing aseptically the crust.

The other experimental bread samples (B\_BROC; B\_CAUL; B\_ART; B\_FEN; B\_ZUC; B\_MUSH) were prepared by adding 15% of the respective vegetable flours (F\_BROC; F\_CAUL; F\_ART; F\_FEN; F\_ZUC; F\_MUSH) to 930 g of mixture flour (corn starch, rice flour, potato starch) (named as F\_CTRL). Before dough making, each vegetable powder was hydrated with an appropriate quantity of water to the desired consistency. All the experimental samples were realized using the same procedure as for the B\_CTRL. For chemical analyses, the slices were dried at 35°C in a ventilated stove (BINDER GmbH, Tuttlingen, Germany), milled and sieved in order to achieve a fine bread dust (800 µm).

### 2.3. Bread Sensory Analysis

Bread sensory properties were carried out with a panel of 8 trained tasters. Before sensory analysis, samples were sliced with an electric slicing knife (thickness of 15 mm) (Atlantic, Calenzano, Italy) without removing the crust. Samples were placed on white plates and identified with random three-digit numbers. Bread sensorial quality was determined using a scale from 1 (extremely unpleasant), to 9 (extremely pleasant) based on overall acceptance (color, appearance, odor, taste, crust, crumb firmness and presence of large bubbles).

### 2.4. Chemical Analyses

For chemical analysis, bioactive compounds were extracted in triplicate from samples using a method described by Lim, Park, Ghafoor, Hwang, and Park [8]. Briefly, 1 g of sample powder was homogenized in 10 ml of aqueous ethanol (80%; v/v) for 2 h at 37°C and centrifuged at 12,000 g for 15 min. The supernatant was collected and stored at 4°C prior to analysis.

#### 2.4.1. Total Phenols, Flavonoids and Antioxidant Activity Determination

Total phenolic compounds were determined by UV-vis spectrophotometric method according to Lim *et al.* [8] and using gallic acid as standard. The total phenolic content was expressed as mg gallic acid/g of dry weight (dw).

Total flavonoid content was determined spectrophotometrically using the method of Marinelli, Padalino, Nardiello, Del Nobile, and Conte [9] based on aluminum chloride colorimetric method and using quercetin as standard. The total amount of flavonoids was expressed as mg of quercetin/g of dry weight (dw).

The antioxidant activity was evaluated using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay according to the method of Cerdola, Cardinali, Del Nobile, and Conte [10] and using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as standard. The results were ex-

pressed as mg Trolox equivalents for gram of dry weight (dw).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured according to method of Das *et al.* [7] with slight modifications. Bread extracts (100 µl) were mixed with 250 µL of DPPH ethanolic solution (1 mmol/l) and 2.0 mL of 80% ethanol, shaken vigorously and allowed to stand in darkness at room temperature for 20 min. The absorbance was measured at 517 nm in a spectrophotometer (UV1800, Shimadzu Italia s.r.l., Milano, Italy), using 80% ethanol as blank. The control solution was constituted by 80% ethanol instead of the sample. The radical scavenging activity was expressed as the inhibition percentage using the Equation (1), where  $A_{\text{control}}$  and  $A_{\text{sample}}$  are the absorbance of the control solution and the absorbance of the sample solution, respectively:

$$\% \text{ Inhibition of DPPH} = A_{\text{control}} - A_{\text{sample}} \cdot 100 / A_{\text{control}} \quad (1)$$

#### 2.4.2. Available Carbohydrates Determination and *In vitro* Digestion

The available carbohydrates (ACH) were determined according to the method described in the “Available Carbohydrates and Dietary Fibre” assay kit (Megazyme).

For *in vitro* digestion, based on the methodology of Mastromatteo *et al.* [11], the results were expressed as mg of glucose equivalent (GE) per g of sample vs. time (0 - 180 min). Anyway, glycemic index was calculated as the incremental area under the curve (iAUC) for the tested products divided by the iAUC of a reference food (white bread) [12].

### 2.5. Statistical Analysis

All the experimental data were subjected to statistical evaluation using a one-way variance analysis (ANOVA). The mean values were compared and grouped by Fisher’s least significant difference test at a significance level of  $p < 0.05$  using STATISTICA 7.1 for Windows (StatSoft, Inc., Tulsa, OK, USA).

## 3. Results and Discussion

### 3.1. Bread Sensory Evaluation

Results of bread sensory quality are reported in **Table 1**. Data revealed that all sensory scores were in the range 5 - 8 indicating that breads were acceptable, with some differences among them. In particular, the crumb color of B\_ART sample had the lowest score, since the color of the artichoke flour was dark green and interfered with the original color of the control bread. The second lower value was for sample B\_ZUC, in fact, also in this case the green color of crumb was less appreciated than the other samples. Specifically, the B\_FEN and B\_CAUL samples had the highest values, since the corresponding flours had a color that did not interfere with the typical light color of the control bread. For other sensory characteristics (odor and taste), B\_FEN sample had the highest value, B\_ART was positively assessed, while B\_BROC and B\_ZUC samples showed the lowest values, due to the perception of a sensation described by the

**Table 1.** Sensory characteristics of bread samples.

Sample	Color	Appearance	Odor	Taste	Crust firmness	Crumb firmness	Large bubbles	Overall quality
B_CTRL	7.44 ± 0.32 <sup>b</sup>	7.50 ± 0.27 <sup>b</sup>	7.25 ± 0.38 <sup>b</sup>	7.13 ± 0.23 <sup>b</sup>	7.44 ± 0.18 <sup>b</sup>	7.38 ± 0.23 <sup>a,b</sup>	7.56 ± 0.42 <sup>a,b</sup>	7.50 ± 0.38 <sup>b</sup>
B_BROC	7.38 ± 0.35 <sup>b</sup>	7.13 ± 0.23 <sup>b,c</sup>	6.75 ± 0.27 <sup>c</sup>	6.06 ± 0.32 <sup>d</sup>	7.19 ± 0.26 <sup>b,c</sup>	6.69 ± 0.37 <sup>c</sup>	7.00 ± 0.38 <sup>c</sup>	6.50 ± 0.27 <sup>c</sup>
B_CAUL	7.69 ± 0.37 <sup>a,b</sup>	6.88 ± 0.23 <sup>c</sup>	6.81 ± 0.46 <sup>c</sup>	6.25 ± 0.27 <sup>d</sup>	6.50 ± 0.27 <sup>d</sup>	6.56 ± 0.42 <sup>c</sup>	6.31 ± 0.37 <sup>d</sup>	6.38 ± 0.23 <sup>c,d</sup>
B_ART	6.25 ± 0.27 <sup>c</sup>	6.50 ± 0.38 <sup>d</sup>	7.38 ± 0.23 <sup>b</sup>	6.56 ± 0.32 <sup>c</sup>	6.63 ± 0.23 <sup>d</sup>	5.56 ± 0.32 <sup>d</sup>	6.25 ± 0.38 <sup>d</sup>	6.13 ± 0.44 <sup>d</sup>
B_FEN	7.94 ± 0.32 <sup>a</sup>	8.06 ± 0.18 <sup>a</sup>	8.13 ± 0.23 <sup>a</sup>	7.56 ± 0.18 <sup>a</sup>	7.81 ± 0.26 <sup>a</sup>	7.69 ± 0.26 <sup>a</sup>	7.75 ± 0.27 <sup>a</sup>	7.88 ± 0.35 <sup>a</sup>
B_ZUC	6.75 ± 0.27 <sup>b</sup>	5.50 ± 0.27 <sup>e</sup>	5.69 ± 0.37 <sup>d</sup>	5.31 ± 0.37 <sup>e</sup>	6.56 ± 0.42 <sup>d</sup>	5.00 ± 0.46 <sup>e</sup>	5.06 ± 0.32 <sup>e</sup>	5.13 ± 0.23 <sup>e</sup>
B_MUSH	7.50 ± 0.38 <sup>b</sup>	7.19 ± 0.26 <sup>b</sup>	6.81 ± 0.37 <sup>c</sup>	6.13 ± 0.23 <sup>d</sup>	7.13 ± 0.35 <sup>c</sup>	7.25 ± 0.38 <sup>b</sup>	7.31 ± 0.26 <sup>b,c</sup>	6.56 ± 0.18 <sup>c</sup>

<sup>a-e</sup>Means (±SD, n = 3) in the same column followed by different superscript letters differ significantly (p < 0.05).

panelists as “herbaceous”. In addition, it is worth noting that B\_ZUC sample showed a crumb very compact and with a presence of small bubbles, which was most probably due to the lower amount of water added. The second lowest value was for sample B\_ART, while, also in this case, B\_FEN sample showed the highest value, due to a soft and porous crumb with the presence of large bubbles related to the method of hydration of vegetable flour. Results highlighted that the maximum score of overall quality (more than 7) was obtained by the B\_FEN sample, and there were statistically significant differences compared to all other samples including the control. In terms of overall quality, the bread enriched with fennel flour was the most appreciated, since this sample showed the higher scores for all the sensory attributes evaluated.

## 3.2. Chemical Quality

### 3.2.1. Total Phenols, Flavonoids and Antioxidant Activity of Flour and Bread

The total phenolic compounds of flour and bread were expressed as mg gallic acid per gram of dry weight and shown in **Table 2**. The F\_ART sample had the highest phenolic content (26.51 ± 1.92 mg/g dw) followed by F\_BROC (8.22 ± 0.14 mg/g dw) and F\_ZUC samples (7.80 ± 0.13 mg/g dw). Among the vegetable flours, samples F\_CAUL, F\_MUSH and F\_FEN had the lowest phenolic content (6.43 ± 0.27 mg/g dw, 6.11 ± 0.53 mg/g dw and 4.54 ± 0.08 mg/g dw, respectively) and the differences between them and the control flour (0.29 ± 0.00 mg/g dw) were statistically significant. With respect to bread, the phenolic value was higher in B\_ZUC sample (2.58 ± 0.02 mg/g dw) followed by B\_ART (2.50 ± 0.01 mg/g dw), even if F\_ART sample showed a value much higher compared to all the other flours. The results were also high, surprisingly, for B\_CAUL (2.45 ± 0.08 mg/g dw) and B\_FEN samples (2.45 ± 0.03 mg/g dw), followed by B\_BROC sample (2.44 ± 0.04 mg/g dw). In addition, consistent with the above results, the content of phenols was lower in the B\_CTRL sample (0.26 ± 0.01 mg/g dw).

Results showed significant differences between control bread and bread with vegetable flours, furthermore, the total phenolic content in breads was in all cases lower than in respective flour. Polyphenols are highly unstable species that are

**Table 2.** Total phenolic content, total flavonoids content and antioxidant activity of flour and bread samples.

Sample	Antioxidant activity			
	Total phenolic content (mg gallic acid g <sup>-1</sup> dw)	Total flavonoids content (mg quercetin g <sup>-1</sup> dw)	ABTS (mg Trolox g <sup>-1</sup> dw)	DPPH (% Inhibition)
F_CTRL	0.29 ± 0.00 <sup>e</sup>	0.14 ± 0.02 <sup>f</sup>	0.26 ± 0.02 <sup>d</sup>	3.94 ± 0.10 <sup>f</sup>
F_BROC	8.22 ± 0.14 <sup>b</sup>	6.61 ± 0.27 <sup>b</sup>	8.58 ± 0.33 <sup>b</sup>	80.94 ± 0.77 <sup>b</sup>
F_CAUL	6.43 ± 0.27 <sup>c</sup>	3.83 ± 0.58 <sup>c,d</sup>	7.61 ± 0.26 <sup>b</sup>	79.58 ± 0.63 <sup>b</sup>
F_ART	26.51 ± 1.92 <sup>a</sup>	26.43 ± 1.93 <sup>a</sup>	31.56 ± 2.28 <sup>a</sup>	91.26 ± 0.48 <sup>a</sup>
F_FEN	4.54 ± 0.08 <sup>d</sup>	2.04 ± 0.13 <sup>e</sup>	5.90 ± 0.42 <sup>c</sup>	69.00 ± 3.01 <sup>c</sup>
F_ZUC	7.80 ± 0.13 <sup>b</sup>	4.56 ± 0.52 <sup>c</sup>	8.59 ± 0.33 <sup>b</sup>	47.27 ± 2.95 <sup>e</sup>
F_MUSH	6.11 ± 0.53 <sup>c</sup>	2.48 ± 0.31 <sup>d,e</sup>	7.81 ± 0.16 <sup>b</sup>	61.31 ± 0.42 <sup>d</sup>
B_CTRL	0.26 ± 0.01 <sup>d</sup>	0.03 ± 0.00 <sup>d</sup>	0.13 ± 0.01 <sup>e</sup>	0.01 ± 0.02 <sup>d</sup>
B_BROC	2.44 ± 0.04 <sup>b,c</sup>	0.57 ± 0.04 <sup>b</sup>	0.71 ± 0.13 <sup>b,c</sup>	5.73 ± 0.18 <sup>a</sup>
B_CAUL	2.45 ± 0.08 <sup>b,c</sup>	0.45 ± 0.02 <sup>c</sup>	0.68 ± 0.07 <sup>c</sup>	4.22 ± 0.28 <sup>b</sup>
B_ART	2.50 ± 0.01 <sup>b</sup>	0.70 ± 0.03 <sup>a</sup>	0.46 ± 0.02 <sup>d</sup>	5.91 ± 0.12 <sup>a</sup>
B_FEN	2.45 ± 0.03 <sup>b,c</sup>	0.42 ± 0.03 <sup>c</sup>	0.56 ± 0.08 <sup>c,d</sup>	4.46 ± 0.23 <sup>b</sup>
B_ZUC	2.58 ± 0.02 <sup>a</sup>	0.57 ± 0.05 <sup>b</sup>	1.03 ± 0.16 <sup>a</sup>	3.54 ± 0.35 <sup>c</sup>
B_MUSH	2.40 ± 0.04 <sup>c</sup>	0.45 ± 0.06 <sup>c</sup>	0.87 ± 0.16 <sup>a,b</sup>	4.55 ± 0.24 <sup>b</sup>

<sup>a-f</sup>Means (±SD, n = 3) in the same column followed by different superscript letters differ significantly (p < 0.05).

subject to numerous reactions in the course of food processing [13]. For baked products, such as bread, it is possible the occurrence of the degradation and loss of phenolic compounds due to the heat and oxidation during the mixing and baking process [14]. Some studies have reported the loss of antioxidants during mixing dough, knead and bake, arguing that the antioxidant active compounds present in flours might be damaged or degraded as a consequence of the heat/thermal process during baking [15]. In addition, Samaras, Camburn, Chandra, Gordon, and Ames [16] argued that the products of Maillard reaction during baking may be involved in the estimation of phenolic compounds, because caramelization products and polyphenolic oxidation products may have antioxidant activity but can also act as false positives in the estimation. In accordance with our results, also Holtekjølen *et al.* [14], Leenhardt *et al.* [15] and Chlopicka, Pasko, Gorinstein, Jedryas, and Zagrodzki [17] showed that the contents of phenols were lower in the experimental breads than in the respective flour. Han and Koh [18] observed a reduction of phenolic acids levels of about 20% - 30% in breads in comparison to start material-enriched flour. In addition, they also showed that phenolics responsible for the antioxidant potential of enriched breads are already strongly bound to bread components at the stage of forming the bread mix and mixing the dough. Specifically, Leenhardt *et al.* [15] observed a loss of antioxidants during dough mixing and kneading. Other researchers suggested that antioxidant activity of breads could be modified by active oxidative enzymes presented in ingredients of compounds used in breads production [7]. Some studies have been performed to determine the impact of

the bread-making process on the lipid-soluble antioxidant content of wheat and to understand the mechanisms responsible for the loss of these compounds; anyhow, a few strategies have been developed to preserve antioxidant compounds in bread [15].

**Table 2** also shows the total flavonoids content in flour and bread, expressed as mg quercetin per gram of dry weight. The results showed that, as for the total phenols, also the content of total flavonoids was much higher in the F\_ART sample ( $26.43 \pm 1.93$  mg/g dw) followed by F\_BROC ( $6.61 \pm 0.27$  mg/g dw) and F\_ZUC ( $4.56 \pm 0.52$  mg/g dw). The lowest value of flavonoids was detected in sample F\_CTRL ( $0.14 \pm 0.02$  mg/g dw) and the differences were statistically significant among all samples. In all cases, the content of total flavonoids in flours was higher than in the breads. Specifically, the B\_ART sample showed the greatest loss of flavonoids content compared to the corresponding flour. However, this sample showed the highest content of flavonoids ( $0.70 \pm 0.03$  mg/g dw) respect to the breads with addition of the other vegetable flours. Moreover, flavonoids content was lower in B\_CTRL sample ( $0.03 \pm 0.00$  mg/g dw) and also in this case the results showed significant differences between control bread and bread with vegetable flours. According to the results presented, breads showed a much higher loss of flavonoids than the polyphenols, this may be due to the greater sensitivity of flavonoids at high baking temperatures. Thermal processing is generally considered to be destructive to nutrients, because most bioactive compounds become unstable when exposed to heat [19].

Comparing the flour types with the corresponding bread samples after the baking process, it is worth noting that the bread-making process created a loss of total flavonoids content. The low level of extractable bioactive compounds from bread after baking might be a result of formation of phenolic-containing complexes with polysaccharides and/or proteins/enzymes [20]. Sivam, Sun-Waterhouse, Perera, and Waterhouse [21] confirmed the existence of interactions between phenolics (especially flavonoids) and protein. In particular, flavonoids also interact with starch [22]. Zhang, Yang, Li, and Gao [23] showed that the solid complex of starch with quercetin leads to a reduction in starch digestibility via formation of resistant starch. These interactions may significantly influence the bioactivity of phenolic antioxidants and the bioaccessibility of starch and proteins in *in vivo* conditions.

In order to test the antioxidant activity of flour and bread two methods were used, ABTS and DPPH assays; for both, results are shown in **Table 2**. According to data obtained by ABTS assay, expressed as mg Trolox per gram of dry weight, as for phenols and flavonoids, also in this case F\_ART sample showed significantly higher antioxidant activity ( $31.56 \pm 2.28$  mg/g dw) compared to all the other vegetable flours. In fact, Alonso, Guillen, Barroso, Puertas, and Garcia [24] reported that there is a positive correlation between total polyphenolic content and sample antioxidant activity. The second highest values were for F\_ZUC ( $8.59 \pm 0.33$  mg/g dw) and F\_BROC samples ( $8.58 \pm 0.33$  mg/g dw), while, among the vegetable flours, the sample F\_FEN had the lowest antioxidant ac-

tivity ( $5.90 \pm 0.42$  mg/g dw). Also in this case, F\_CTRL sample had the lowest antioxidant activity ( $0.26 \pm 0.02$  mg/g dw). However, according to the data obtained, surprisingly, for bread with vegetable flours, the sample B\_ART had the lowest antioxidant activity ( $0.46 \pm 0.02$  mg/g dw), while, B\_ZUC sample, consistently with the above results, showed the highest value ( $1.03 \pm 0.16$  mg/g dw). These results could be explained, firstly, due to the considerable loss of the polyphenolic and flavonoid compounds, which has occurred among the artichoke flour and the corresponding bread, compared to all the other samples. Secondly, it is possible that the antioxidant activity of bread with the addition of zucchini flour was better reflected by the ABTS test compared to the bread with artichoke. Finally, B\_CTRL sample showed the lowest antioxidant activity ( $0.13 \pm 0.01$  mg/g dw) with significant differences among all the bread samples.

DPPH assay was used as an additional method to determine the antioxidant activity of flour and bread samples. Specifically, the antioxidant capacity of the samples was evaluated based on scavenging capacity of free radical DPPH<sup>•</sup>. The antioxidant activity determined by DPPH test for flour and breads was expressed as percentage inhibition or percentage of discoloration. Antioxidant activity was highest in sample F\_ART ( $91.26\% \pm 0.48\%$ ) followed by F\_BROC ( $80.94\% \pm 0.77\%$ ), F\_CAUL ( $79.58\% \pm 0.63\%$ ) and F\_FEN samples ( $69.00\% \pm 3.01\%$ ). Surprisingly, F\_ZUC sample had the lowest antioxidant activity ( $47.27\% \pm 2.95\%$ ).

For bread, the results largely showed the same trend of the flour, the antioxidant activity was higher in B\_ART ( $5.91\% \pm 0.12\%$ ) and B\_BROC samples ( $5.73\% \pm 0.18\%$ ), while the sample B\_ZUC showed the lowest antioxidant activity ( $3.54\% \pm 0.35\%$ ). In addition, F\_CTRL and B\_CTRL samples had lower values of antioxidant activity with significant differences compared to all the other samples.

All these results suggest that there was a different trend in the antioxidant capacity of bread between ABTS and DPPH, but a similar one in the flours. Antioxidant capacity detected by ABTS test was significantly higher for bread with addition of zucchini flour compared to the value obtained with DPPH test. On the contrary, the DPPH assay showed a high antioxidant activity in bread with artichoke flour. Floegel, Kim, Chung, Koo, and Chun [25] reported that the correlation between the antioxidant capacity detected by ABTS and DPPH tests was higher in beverages and fruit but lower in vegetables. In particular, Kim, Lee, Lee, and Lee [26] described the difference between the two methods, specifying that the ABTS test is based on the generation of a blue/green color ABTS<sup>•+</sup>, which is applicable to both hydrophilic and lipophilic antioxidant systems; while DPPH test uses a radical dissolved in organic media and it is applicable to hydrophobic systems. Furthermore, Chlopicka *et al.* [17] reported that the DPPH method is based on the evaluation of reducing ability of antioxidants to DPPH<sup>•+</sup>, which is stable radical nitrogen, holding an odd-electron, therefore, steric accessibility is a determining factor of the analytical reaction. This explains why the DPPH assay is more sensitive to small molecules that have a good access to the radical site, and is less sensitive to larger molecules.

Certainly, further studies will be necessary to deepen these issues, in order to establish the different composition and stability of the phenolic compounds present in the different vegetable flours. Finally, it can be noted that despite the loss of antioxidant compounds, during the baking processes, the bread samples enriched with vegetable flours showed a significant amount of polyphenolic and flavonoid compounds, with a higher antioxidant activity than in the control bread.

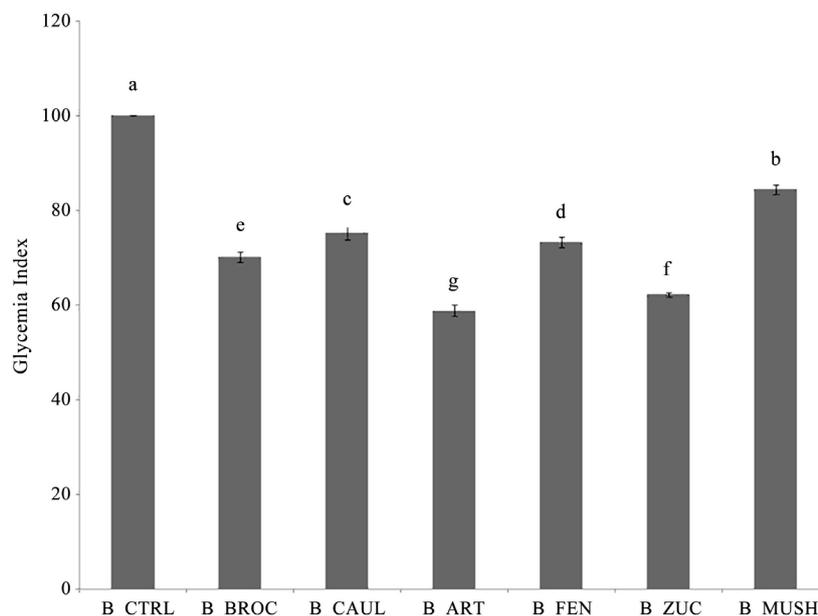
### 3.2.2. Glycemic Response and Available Carbohydrates Content of Bread Samples

**Table 3** shows the glucose released during digestion for the investigated samples. As can be seen, a rapid release and significant differences of glucose were observed at 20, 60 and 120 min following the addition of pancreatin and amyloglucosidase. In particular, the amount of glucose released from B\_ART and B\_ZUC samples was statistically lower than that of B\_CTRL and the other samples. Anyway, at the end of the digestion (180 min) the B\_CTRL sample recorded a highest release of glucose ( $376.9 \pm 7.10$  mg GE/g bread). This behavior is reflected on the glycemic index determined on vegetable flour-fortified bread and B\_CTRL samples (**Figure 1**). In fact, the incorporation of the vegetable flour at 15% caused a statistically significant decrease in glycemic index (GI) compared to the control sample, thereby contributing to elevate nutritional value of bread. Specifically, B\_ART and B\_ZUC samples recorded a lowest glycemic index ( $59 \pm 1.21$  and  $62 \pm 0.49$ , respectively), thus confirming their content of glucose equivalent obtained at the end of the digestion process ( $303.7 \pm 14.7$  and  $295.4 \pm 9.80$  mg GE/g bread, respectively). It is conceivable that these results could be due to high dietary fiber content of vegetable flours used to enrich bread. In fact, Petitot, Barron, Morel, and Micard [27] have demonstrated that inclusion of dietary fibers would reduce blood glucose responses because of a slower absorption of glucose from the gastrointestinal tract, favoring lower susceptibility of starch to digestive enzymes. Finally, these finding appear to be related to the available carbohydrate content of the bread samples investigated (**Figure 2**). In fact, also in this case, B\_ART and B\_ZUC samples recorded the lowest ACH value content among samples ( $58.2 \pm 2.79$  and  $60.4 \pm 0.20$  g ACH/100 g bread, respectively).

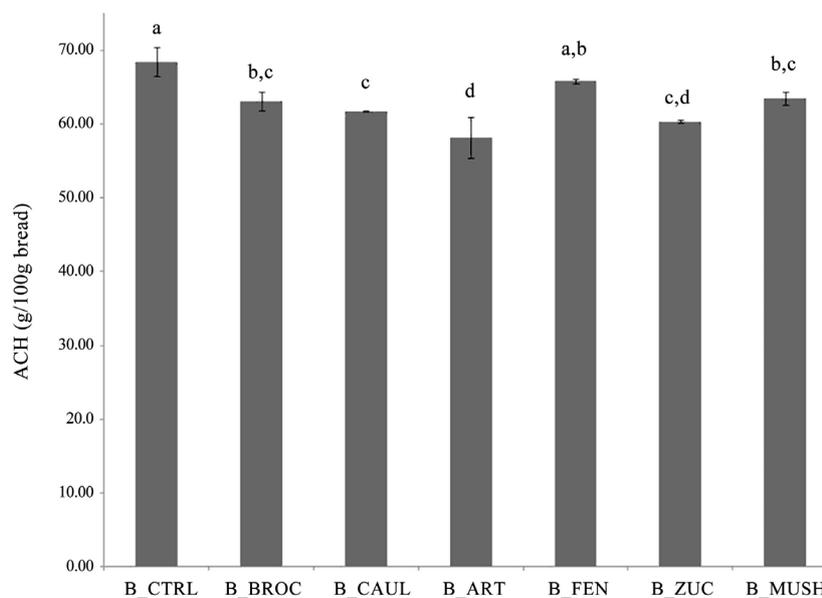
**Table 3.** Determination of glucose release of bread samples.

Digestion time (min)	Glucose equivalent (mg/g bread)						
	B_CTRL	B_BROC	B_CAUL	B_ART	B_FEN	B_ZUC	B_MUSH
0	$33.4 \pm 1.50^a$	$11.0 \pm 0.00^f$	$20.5 \pm 2.70^d$	$16.0 \pm 0.07^e$	$27.8 \pm 0.90^c$	$21.6 \pm 2.00^d$	$9.30 \pm 0.00^f$
20	$108.9 \pm 5.60^a$	$73.6 \pm 6.00^c$	$84.0 \pm 7.60^b$	$58.7 \pm 4.80^d$	$88.7 \pm 6.70^b$	$54.7 \pm 2.70^d$	$116.1 \pm 2.90^a$
60	$229.1 \pm 15.6^a$	$147.7 \pm 10.5^b$	$149.8 \pm 7.60^b$	$109.6 \pm 7.00^c$	$156.1 \pm 5.90^b$	$105.4 \pm 7.80^c$	$231.6 \pm 3.20^a$
120	$350.2 \pm 2.40^a$	$238.7 \pm 5.80^b$	$264.4 \pm 10.3^b$	$188.9 \pm 6.80^c$	$242.3 \pm 15.0^b$	$157.7 \pm 10.1^d$	$266.4 \pm 17.9^b$
180	$376.9 \pm 7.10^a$	$307.6 \pm 14.5^{c,d}$	$320.1 \pm 13.4^{b,c}$	$303.7 \pm 14.7^{c,d}$	$315.5 \pm 3.20^c$	$295.4 \pm 9.80^d$	$338.6 \pm 6.20^b$

<sup>a-f</sup>Means ( $\pm$ SD, n = 3) in the same row followed by different superscript letters differs significantly ( $p < 0.05$ ).



**Figure 1.** Glycemic response of bread samples: control bread (B\_CTRL); broccoli flour bread (B\_BROC); cauliflower flour bread (B\_CAUL); artichoke flour bread (B\_ART); fennel flour bread (B\_FEN); zucchini flour bread (B\_ZUC); mushroom flour bread (B\_MUSH). Bars represent standard error of means (n = 3) and means with different letters are significantly different ( $P < 0.05$ ).



**Figure 2.** Available carbohydrates content (ACH) of bread samples: control bread (B\_CTRL); broccoli flour bread (B\_BROC); cauliflower flour bread (B\_CAUL); artichoke flour bread (B\_ART); fennel flour bread (B\_FEN); zucchini flour bread (B\_ZUC); mushroom flour bread (B\_MUSH). Bars represent standard error of means (n = 3) and means with different letters are significantly different ( $P < 0.05$ ).

#### 4. Conclusion

The results obtained in this study indicated that the enrichment of gluten-free

bread with different types of vegetable flours allowed the creation of bread with enhanced functional properties without adversely affecting the technological parameters. Results indicated that fennel flour gives the best results among the tested vegetable flours from a sensory point of view. Specifically, the B\_FEN sample improved satisfactory all sensory bread properties and it showed the higher scores with statistically significant differences compared to all the other samples, including also the control bread. As regards chemical composition, all the enriched bread samples showed very good functional properties respect to the control sample. In particular, B\_ART and B\_ZUC samples have high content of phenolic compounds, though they need to be improved from the sensory point of view. The GI of all enriched bread samples was lower respect to the control sample, and also in this case, B\_ART and B\_ZUC were the best samples. These results indicate that vegetable flours can be effectively incorporated in gluten-free bread to improve the functional properties, and reduce the glycemic index without affecting the sensory quality.

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