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Maca flour: a powerful ingredient for functionally enhanced bread

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Abstract

In this work a new functionally enhanced food was prepared by using Maca flour as powerful ingredient. Bread with improved antioxidant and anti-inflammatory properties was prepared and characterized by performing different assays. For the characterization of Maca enhanced bread, in vitro tests, such as 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Oxygen radical absorbance capacity (ORAC), Folin-Ciocalteu and Deoxyribose assays were performed in order to evaluate the antioxidant properties, while the anti-inflammatory activity was investigated in terms of peroxynitrite scavenging ability. Furthermore, *in vitro* enzymatic assays were performed in the aim to evaluate the efficiency of the proposed functional food in the reduction of sugar intake. The obtained data confirmed the high potential applicability of prepared bread in human nutrition.

Introduction

Functional foods are defined as any substance or component of a food that offers health benefits in the prevention and treatment of diseases. The main functional components of foods are fibers, polyunsaturated fatty acids, phytochemicals, active peptides, prebiotics, probiotics and antioxidant compounds (Kucuk, 2002). Recently, antioxidant compounds have been viewed as an important class of food ingredients that can be added to introduce extra health benefits to various food products. Applications of antioxidants in food production have included the production of value-added products such as dietary supplements for disease prevention (Shrikhande, 2000; Santos Calderelli *et al*., 2010).

Nutritional studies have recently shown that a regular consumption of polyphenolic antioxidants, contained in fruits, vegetables and their related juices, has a positive effect in the treatment and prevention of a wide range of pathologies, including cancer (Shankar *et al*., 2007), stroke (Neto, 2007), coronary heart disease (Widlansky *et al*., 2007; Khan *et al*., 2007) and neurodegenerative disease, such as Alzheimer's disease (Mandel *et al*., 2004; Bonfili *et al*., 2008). These compounds are bioactive secondary plant metabolites that are widely present in commonly consumed foods of plant origin (Repo-Carrasco-Valencia *et al*., 2010).

In literature, several examples of the breadmaking use of Andean Crops are reported. In particular, the effect of addition of flours from the highly nutritious Andean crops quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*), kiwicha (*Amaranthus caudatus*), and tarwi (*Lupinus mutabilis*) were investigated in wheat doughs and fresh bread quality (Rosell *et al*., 2009). Up to 20% replacement of wheat flour by quinoa or kiwicha has been reported (Bruemmer *et al*., 1992; Chauhan *et al*., 1992; Morita *et al*., 2001) and a wheat flour replacement by 10% tarwi have been founded to give acceptable bread quality (Gross *et al*., 1983; Lorenz *et al*., 1991).

Among the different plant materials showing interest in biomedical field, our attention was focused on Maca (*Lepidium meyenii*), an annual or biennial herbaceous plant native to the Americas. This cruciferous vegetable belongs to the family of Brassicaceae and grows in the central Andes of Peru at an altitude greater than 3500 m above the sea level. Several ecotypes can be distinguished in this cultivation area according to the color of their roots, such as yellow, purple, white, grey, black, yellow/

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purple and white/purple (Gonzales *et al*., 2006; Wang *et al*., 2007). Maca tuber is widely used as a food ingredient for human consumption, due to its nutritional value and phytochemical content (Dini *et al*., 1994), but it is also employed in traditional medicine in the aim to enhance sexual drive and female fertility in human beings and domesticated animals. The oral administration of a lipid extract of Maca, indeed, increased the sexual function of mice and rats (Zheng *et al.*, 2000), while Sandoval *et al.* (2002) reported on the ability of this plant to scavenge free radicals and protect cells against oxidative stress. Maca is also characterized by other claimed activities such as the reduction of the effects of stress, including combating the increase in corticosterone and all the parameters related to this increase, and the reduction in free fatty-acids and glucose levels in plasma produced by stress (Lopez-Fando *et al.*, 2004). Maca powder has also been shown to exhibit an anti-tiredness effect, and can increase the duration of mobility in forced swimming tests of mice (Rubio *et al*., 2006; Yu *et al.*, 2004). Furthermore, Maca meal supplementation increased food intake, growth and feed utilization along with improving survival in rainbow trout juveniles (Lee *et al.*, 2004; Lee *et al.*, 2005) and this effect could be explained with the stimulation of growth hormone production. This plant has been used to treat women with menopausal symptoms because it was found to increase calcium content in the femur of the rats (Zhang *et al*., 2006) and, thus, to overcome the reduction of bone mineral density.

Based on these considerations, in this paper a new functional food was prepared by using Maca flour. Bread with enhanced antioxidant and anti-inflammatory properties was prepared and characterized, showing high potential applicability in human nutrition. For the characterization of Maca enhanced bread, in vitro tests such as 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Oxygen radical absorbance capacity (ORAC), Folin-Ciocalteu and Deoxyribose assays were performed to evaluate the antioxidant properties, while the anti-inflammatory activity was investigated in terms of peroxynitrite scavenging ability. Finally, *in vitro* enzymatic assays were performed to determine the efficiency of the proposed functional food in the reduction of sugar intake.

Materials and Methods

Materials

Wheat and Maca Flours were provided by Principium Europe Srl, (Milan, Italy). Folin-Ciocalteu reagent, sodium carbonate, gallic acid, 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, deoxyribose, $FeCl_3$, hydrogen peroxide (30% w/w), ethylenediaminetetraacetic acid disodium salt (EDTA), dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium buffer saline (PBS), ascorbic acid, thiobarbituric acid, trichloroacetic acid, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), fluorescein, 2,2'-azobis-(2-amidinopropane) dihydrochloride (AAPH), sodium nitrite, hydrochloric acid (37% w/w), MnO_2, α-amylase (EC 3.2.1.1), sodium potassium tartrate, sodium hydroxide, 3,5-dinitrosalicylic acid (DNS), α-glucosidase (EC 3.2.1.20), sodium acetate, o-dianisidine (DIAN), Glucose oxidase/peroxidase reagent (PGO), perchloric acid (70% ww) were obtained from Sigma-Aldrich (Sigma Chemical Co., St Louis, MO, USA). All used solvent were HPLC-grade and provided by Fluka Chemika-Biochemika (Buchs, Switzerland).

Breadmaking process

Wheat-Maca flour blends (0, 5, 10, 15, 20%,) were used for making small pan breads. Blended flours (300 g) were mixed with water (59% v/w, blend basis), salt (1.8% w/w, blend basis) and yeast (2% w/w, blend basis) in a 300-g Brabender farinograph bowl for 8 min. After kneading, the doughs were divided into 50-g pieces and individually placed into aluminum pans. Fermentation was done in a proofing cabinet for 45 min at 30°C and 85% RH. The dough pieces were baked for 35 min at 165°C in an electric oven and the loaves were removed from the pans and cooled at room temperature for 30 min. Maca breads were named MB.

Evaluation of total phenolic compounds by Folin-Ciocalteu procedure

Amount of total phenolic equivalents was determined using Folin-Ciocalteu reagent procedure, according to the literature with some modifications (Puoci *et al*., 2011). 100 mg of each sample were dispersed in distilled water (6 mL) in a volumetric flask. Folin-Ciocalteu reagent (1 mL) was added and the contents of flask were mixed thoroughly. After 3 min, 3 mL of sodium carbonate (2%) were added, and then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm against a control prepared using wheat bread under the same reaction conditions. The amount of total phenolic compounds in each bread sample was

expressed as gallic acid equivalent concentration using a calibration curve recorded by employing five different gallic acid standard solutions. 0.5 mL of each solution were added to the Folin-Ciocalteu system to raise the final concentration of 8.0, 16.0, 24.0, 32.0, and 40.0 μM, respectively. After 2 h, the absorbance of the solutions was measured to record the calibration curve and the correlation coefficient (R^2), slope and intercept of the regression equation obtained were calculated by the method of least square. All samples were assayed in triplicate and data expressed as means (± SD).

Determination of scavenging effect on DPPH radical

In order to evaluate the free radical scavenging properties of bread samples, their reactivity towards the lipophilic stable free radical, 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH), was evaluated (Ardestani and Yazdanparast, 2007). For this purpose, 250 mg of each sample were dispersed in 1 mL of distilled water in a volumetric flask (25 mL) and then 4 mL of ethanol and 5 mL of ethanol solution of DPPH (200 μM) were added, obtaining a solution of DPPH with a final concentration of 100 μM. The sample was incubated in a water bath at 25°C and, after 30 min, 1,5 mL of sample, centrifuged for 10 min (10 000 rev/min) in an ALC microcentrifugette 4214, were used for the DPPH colorimetric determination with a Jasco V-530 UV/Vis spectrometer at a wavelength of 517 nm. The scavenging activity, referable to a decrease in absorbance of the DPPH, was expressed as inhibition (%) calculated according the following equation (1):

$$Inhibition\ (\%) = \frac{A_0 - A_1}{A_0} \times 100 \qquad (1)$$

where A_0 is the absorbance of a standard prepared in the same conditions, but without any sample, and A_1 is the absorbance of each bread sample. All samples were assayed in triplicate and data expressed as means (± SD).

Determination of scavenging effect on ABTS radical cation

The scavenging activity towards the hydrophilic ABTS Radical Cation was assessed according to the literature with slight modifications (Re *et al*., 1999; Kim *et al*., 2002). ABTS was dissolved in water to a 7 mM concentration ABTS, radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Because ABTS and potassium persulfate react stoichiometrically at a ratio of 1:0.5, this will result in incomplete oxidation of the ABTS. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The concentration of the resulting blue-green $ABTS^{\bullet+}$ solution was adjusted to an absorbance of 0.970 ± 0.020 at 734 nm. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of bread samples, 250 mg were mixed with 25 ml of ABTS radical solution. The mixture, protected from light, was incubated in a water bath at 37°C for 5 min. The decrease of absorbance at 734 nm was measured at the endpoint of 5 min. The antioxidant activity was expressed as a percentage of scavenging activity on ABTS radical according to equation (1). All samples were assayed in triplicate and data expressed as means (± SD).

Determination of scavenging effect on hydroxyl radical (•OH)

The scavenging effect on hydroxyl radical was evaluated according to the literature (Parisi *et al*., 2010). Briefly, 60 mg of each sample were dispersed in 0.5 mL of 95% ethanol and incubated with 0.5 mL deoxyribose (3.75 mM), 0.5 mL H_2O_2 (1 mM), 0.5 mL $FeCl_3$ (100 mM), 0.5 mL EDTA (100 mM) and 0.5 mL ascorbic acid (100 mM) in 2.0 mL potassium phosphate buffer (20 mM, pH 7.4) for 60 min at 37 °C. Then samples were filtered and to 1 mL amount of filtrate, 1 mL of thiobarbituric acid (1% w/v) and 1 mL of trichloroacetic acid (2% w/v) were added and the tubes were heated in a boiling water bath for 15 min. The content was cooled and the absorbance of the mixture was read at 535 nm against reagent blank without any sample. The antioxidant activity was expressed as a percentage of scavenging activity on hydroxyl radical according to equation (1). All samples were assayed in triplicate and data expressed as means (± SD).

Oxygen radical absorbance capacity (ORAC)

The antioxidant capacities of sample were evaluated using the method described by Prior and others (Prior *et al*., 2005). The method was carried out using a FLUOstar Optima microplate reader (BMG Labtechnologies, Durham, NC). Clear 48-well (590 μL each) Falcon plates (VWR, St. Louis, MO) were used. 10.0, 25.0, 50.0, 75.0, 100 mg of diluted sample, Trolox standards (6.25, 12.5, 25, 50 μM), and a blank solution (phosphate buffer) were added to each well. The instrument automatically injected 400 μL of fluorescein (0.108 μM) followed by 150 μL of 2,2'-azobis-(2-amidinopropane) dihydrochloride (AAPH) (31.6 mM) to each well. Fluorescence was detected

at 485 nm (excitation) and 520 nm (emission) after the addition of fluorescein and AAPH and every 192 s thereafter for 112 min to allow for a 95% loss of fluorescence. Results were calculated on the basis of differences between the blank, sample (2.0 mg), and standard Trolox curves. A standard curve was generated by plotting the concentrations of Trolox against the area under each curve. ORAC values were calculated according to equation (1). All samples were assayed in triplicate and data expressed as means (± SD).

Determination of scavenging properties on peroxynitrite anion

Peroxynitrite was synthesized from sodium nitrite/H_2O_2 acidified with HCl and the residual H_2O_2 was removed by passing the solution through granular MnO_2. The yellowish stock solution was stored at -80°C and its concentration was evaluated immediately before its use by measuring the absorbance at 302 nm (Schinella *et al*., 2009). The measurements of relative antioxidant capacity were determined by using fluorescein as detecting molecule. Briefly, Fluorescein (to obtain 2 x 10^{-6} mol L^{-1} final) in 100 mM phosphate buffer, pH 7.4, was mixed in the presence or absence of antioxidant with 100 x 10^{-6} mol L^{-1} $ONOO^-$ to the final volume of 2 ml. As reported in literature, the immediate mixing of the sample with the added oxidant is critical for the reproducibility of the assay (Robaszkiewicz *et al.*, 2010). The fluorescence of the samples (100 mg) was measured after incubation at room temperature for 15 min on a Perkin Elmer LS-55 Luminescence spectrometer, equipped with Hamamatsu R928 photomultiplier tube (excitation: 485 nm, emission: 538 nm). The same reaction conditions were applied on the white bread in order to evaluate its interference on peroxynitrite assay. The antioxidant activity was expressed as a percentage of scavenging activity on peroxynitrite radical according to equation (1). All samples were assayed in triplicate and data expressed as means (± SD).

Bioassay for α-amilase inhibition

The α-amylase inhibition assay method was performed according to the literature with some modifications (Fred-Jaiyesimi, 2009). Separate experiments were performed by using white bread and enhanced bread as substrates. 1 mL amount of sample dispersions in PBS (0.5% w/v) buffer (20 mM, pH 7.4) were incubated with 1 mL of α-amylase solution (0.0253 g of α-amylase in 100 mL of cold distilled water). The colorimetric reagent was prepared mixing a sodium potassium tartrate solution (12.0 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2.0M NaOH) and 96 mM DNS solution. The reaction was measured over 3 min. The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, the product being detectable at 540 nm. In the presence of an α-amylase inhibitor less maltose will be produced and the absorbance value would decrease. The α-amylase inhibition was expressed as percentage of inhibition and calculated by equation (1). All samples were assayed in triplicate and data expressed as means (± SD).

Bioassay for α-glucosidase inhibition

The α-glucosidase inhibition was measured through a modified literature method (Kapustka *et al*., 1981) by using white bread and enhanced bread as substrates. Sample suspensions (4% w/v) were prepared by dispersing 12 g of bread in 300 ml of 50 mM sodium acetate buffer. The enzyme solution (EC 3.2.1.20) was prepared by mixing 1 mg of α-glucosidase in 10 ml of ice-cold distilled water. The colorimetric reagent o-dianisidine (DIAN) solution was prepared by dissolving one tablet in 25 ml of distilled water, whilst the peroxidase/glucose oxidase (PGO) system-colour reagent solution was prepared fresh by dissolving one capsule in 100 ml of ice-cold distilled water. In the first step the reaction was started by adding α-glucosidase solution to the sample suspensions and tubes were left to incubate at 37°C for 30 min. After that time, perchloric acid solution (4.2% w/v) was added to stop the reaction. In the second step the generation of glucose was quantified by the reduction of DIAN. The supernatant of tube of step I was mixed with DIAN and PGO and was incubated at 37°C for 30 min. The absorbance of DIAN was measured spectrophotometrically at 500 nm. The α-glucosidase inhibition was expressed as percentage of inhibition and calculated by equation (1). All samples were assayed in triplicate and data expressed as means (± SD).

Results and Discussion

Preparation of Maca breads

Four different enhanced breads were prepared by mixing different amounts of Maca flour with wheat flour and proposed as innovative functional foods. The selected amounts of Maca flour were 5, 10, 15 and 20%, respectively, because a higher amount negatively interferes with the breadmaking process. The so obtained Maca enhanced breads were extensively characterized from a functional point of view to highlight their antioxidant and biological properties and thus evaluate the beneficial effects on

human health.

First of all, a determination of the polyphenol content was performed by specific *in vitro* test, and then the antioxidant activity was evaluated by means of the scavenging activity against free radicals such as DPPH, ABTS, •OH. Furthermore, the ORAC test and the peroxynitrite assay were performed to evaluate the bread efficiency in protecting probe molecules by free radical damage.

Finally, specific enzymatic tests were designed and performed to evaluate the suitability of the proposed functional food in reducing the sugar gastro-intestinal intake.

Evaluation of total phenolic compounds

The determination of the total phenolic compounds was assessed by employing the Folin–Ciocalteu method. This test is related to the reducing properties of a compound/mixture and, although the reducing capacity of a sample is not directly related to its radical scavenging capability, it is a very important parameter of antioxidants. The assay is based on a redox reaction between the phenolic compounds in the sample and a specific reagent which can be summarized as follows (Equation 2):

$$Mo\,(VI)\,(\text{Yellow}) + e^- \rightarrow Mo\,(V)\,(\text{blue}) \quad (2)$$

The resulting phosphomolybdic/phosphotungstic acid complexes ($3H_2O{\bullet}P_2O_5{\bullet}13\ WO_3{\bullet}5\ MoO_3{\bullet}10H_2O$) can be spectrophotometrically detectable at λmax=760 nm *(38)*. For each bread sample, the amount of total phenolic compounds were expressed as mg equivalent of gallic acid and the obtained results are reported in Table 1.

The results clearly show that the cooking process less interferes with the phenolic content of the Maca flour in the different breads, and this is a preliminary confirmation of the suitability of the Andean seeds for the preparation of a functional food.

Table 1. Polyphenol content expressed as gallic acid equivalent (mg GA per g sample)

Sample	GA Equivalent (mg)
Maca Flour	2.13 ± 0.03
Wheat Bread	- - -
MB 5%	0.09 ± 0.01
MB 10%	0.19 ± 0.02
MB 15%	0.25 ± 0.04
MB 20%	0.39 ± 0.07

Determination of scavenging effect on DPPH, ABTS and hydroxyl radicals

In order to evaluate the free radical scavenging properties of bread samples, their reactivity towards a lipophilic stable free radical, such as DPPH, and hydrophilic radicals, such as ABTS and •OH, were evaluated.

DPPH and ABTS are preformed stable organic radical with absorption maximum bands at around 515 and 734 nm, respectively, while the scavenging activity towards •OH was evaluated as the inhibition of its formation using deoxyribose as a substrate. In all the cases, the reaction depends on the hydrogen donating ability of the antioxidant. The scavenger ability of each bread sample was evaluated in term of radical reduction and data are expressed as inhibition (%).

All the samples were found to have high scavenging properties towards the selected radicals (Table 2) and the results are in accordance with the phenolic content of the sample. A higher amount of total phenolic compounds in the sample corresponds to a higher scavenging activity. When comparing the activity of the samples in the different tests, it is clear that the functional compounds in the enhanced breads are more powerful against hydrophilic radicals (•OH inhibition of 51% and ABTS scavenging of 40% by sample MB-20). In all the assays, the activity of Maca flour is retained after the bread preparation, and the slight reduction in the antioxidant efficiency could be referred to the polyphenol degradation in the cooking process.

Table 2. Antioxidant Activity of Maca-Breads expressed as inhibition percentages on DPPH, ABTS, •OH, Peroxynitrite radicals and ORAC values

Sample	Inhibition (%)				
	DPPH	ABTS	·OH	$ONOO^-$	ORAC
Maca Flour	98 ± 2.6	99 ± 2.1	97 ± 1.9	47 ± 1.7	61 ± 0.9
Wheat Bread	2 ± 0.7	3 ± 0.3	3 ± 0.7	4 ± 0.5	2 ± 0.1
MB 5%	3 ± 0.9	6 ± 0.6	8 ± 0.8	7 ± 0.3	6 ± 0.4
MB 10%	9 ± 1.1	16 ± 1.4	20 ± 1.1	12 ± 0.4	10 ± 0.8
MB 15%	15 ± 1.2	29 ± 1.8	27 ± 1.2	20 ± 1.4	16 ± 1.1
MB 20%	21 ± 1.7	40 ± 1.7	51 ± 1.9	25 ± 1.6	21 ± 1.5

ORAC assay

A key requirement for an effective antioxidant is that it must react with free radical before than a biological damage occurs. This parameter can be determined from competition kinetics by measuring the fluorescence decay curve of a probe molecule in the absence and presence of antioxidants, and integrating the area under these curves (Apak *et al*., 2007). A widely accepted test is the oxygen radical absorbance capacity (ORAC) assay (Cao *et al*., 1995) applies a competitive reaction scheme in which antioxidant and substrate kinetically compete for thermally generated peroxyl radicals through the decomposition of azo compounds. The net area under curve (AUC), found by subtracting the AUC of blank from that of antioxidant-containing sample (the fluorescence decay of which is retarded), is an

indication of the total antioxidant concentration of the sample in the ORAC method. The data are expressed as fluorescence decay inhibition (%) (Table 2).

The enhanced breads were found to be able to protect fluorescein by AAPH degradation in a concentration-dependent manner, with MB-20 showing the high efficiency.

Anti-inflammatory activity

In recent studies it was revealed that increased formation of peroxynitrite anion is critically important in the development of thermal hyperalgesia associated with acute and chronic inflammation (Salvemini *et al*., 2001). Thus, the nutraceutical relevance of the proposed Andean flour enriched bread was also evaluated by means of $ONOO^-$ scavenging ability.

This effect was evaluated according to the literature by using fluorescein as a detecting molecular probe and evaluating the fluorescence decay in the presence of newly synthesized anion. As observed for the antioxidant tests, the enhanced breads were able to protect the fluorescein by $ONOO^-$ degradative action (Table 2) with relevant benefits for human tissues. Also in this case, the anti-inflammatory action is in accordance with the polyphenol content.

Enzymatic activity

In recent years, considerable interest has been aroused by antioxidant compounds of natural origin that could play a relevant role in the treatment of several diseases, such as diabetes, cancer, cardiovascular and neurodegenerative pathologies.

In the aim to evaluate the efficiency of enhanced Maca breads in reducing glucose absorption, enzymatic assays were performed. As a monosaccharide, glucose can be readily absorbed from the gastro-intestinal tract into the blood stream after the hydrolysis of glycosidic bonds in digestible carbohydrate foods containing starch, by α-amylase and α-glucosidase enzymes. Inhibition of these enzymes could reduce the high post-prandial blood glucose peaks in diabetics (Cirillo *et al*., 2011).

These enzymes are located on the intestinal brush-border. α-amylases are endoglucanases, which hydrolyze the internal α-1,4 glucosidic linkages in starch, while α-glucosidase hydrolyzes the most important carbohydrates in food, such as starch and sucrose to monosaccharide (e.g. glucose and fructose), which are absorbed into the blood to increase blood glucose values. Usually, these processes take place in the upper portion of the small intestine and greatly increase glucose plasma concentration, especially in diabetic patients. These enzymes have been recognized as therapeutic targets for modulation of postprandial hyperglycemia. Postprandial hyperglycemia is the earliest metabolic abnormality to occur in type 2 diabetes mellitus. Literature studies, indeed, suggested that α-Amylase and α-Glucosidase inhibitors could lengthen the duration time of carbohydrate absorption and flatten the blood-glucose concentrations over time curve (Cirillo *et al*., 2011).

Inhibitory activity of Maca breads were evaluated according to the literature tests by employing, in separate experiments, the bread samples as substrates of the enzymatic reactions. Data are collected in Table 3 and the results show the efficiency of all the samples in reducing the polysaccharide digestion and, thus, the glucose absorption. Furthermore, the inhibitory activity towards glucosidase was found to be higher than that against amylase.

Table 3. Enzymatic activity of Maca-Breads expressed as inhibition percentages on α-amylase and α-glucosidase

Sample	Inhibition (%)	
	α-amylase	α-glucosidase
Maca Flour	36 ± 0.9	48 ± 1.1
Wheat Bread	- - -	- - -
MB 5%	2 ± 0.6	3 ± 0.5
MB 10%	5 ± 0.9	5 ± 0.7
MB 15%	8 ± 1.0	11 ± 0.9
MB 20%	12 ± 1.2	17 ± 1.1

Conclusions

In this work, the applicability of Maca flour, obtained from the annual or biennial herbaceous plant native to the Americas, for the preparation of functional breads with improved biological properties was proved. Four different bread compositions were tested and characterized by specific *in vitro* tests to determine the antioxidant, anti-inflammatory activities, while the ability to reduce the sugar intake was highlighted by performing enzymatic assays using α-amylase and α-glucosidase. The results showed that the biological properties of Maca were retained after the breadmaking process and that the proposed breads are suitable as functional food in human nutrition.

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