Enhancement of biologically active compounds in germinated brown rice and effect of sun-drying

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Abstract

Germinated brown rice (GBR) has been suggested as an approach to mitigate highly prevalent diseases providing nutrients and biologically active compounds. In this study, the content of γ-oryzanol, γ-aminobutyric acid (GABA), total phenolic compounds (TPC) and antioxidant activity of soaked BR (for 24 h at 28°C) and GBR (for 48 and 96 h at 28°C and 34°C) were determined and the effect of sun-drying as an economically affordable process was assessed. Germination improved the content of GABA, TPC and antioxidant activity in a time-dependent manner. Sun-drying increased γ-oryzanol, TPC and antioxidant activity, whereas GABA content fluctuated depending on the previous germination conditions. The main finding of this study indicates that sun-drying is an effective process promoting the accumulation of bioactive compound of GBR. Sun-dried GBR can be consumed as ready-to-eat food after rehydration or included in bakery products to fight non-communicable diseases.

Keywords: Brown rice, sun-drying, germination, γ-aminobutyric acid, γ-oryzanol, phenolic compounds, antioxidant activity.

1. Introduction

Rice (*Oryza sativa* L.) is one of the main cereals produced in the world and the major staple food for almost half of the world population that currently eat rice as staple food. There has been postulated a positive association between white rice intake and risk factors of CVD including metabolic syndrome and type 2 diabetes in low and

middle-income countries (Izadi and Azadbakht, 2015). In recent years, much attention has been paid on the health benefits of brown rice (BR). BR contains health promoting compounds, including dietary fibre, γ-aminobutyric acid (GABA), vitamins, phenolic compounds and γ-oryzanol, that are mainly located in the germ and bran layers which are removed during rice polishing and milling (Monks et al., 2013).

Despite its nutritional value and beneficial physiological effects, BR is not widely consumed because it has poor cooking properties, low organoleptic quality and harsh texture (Burlando and Cornara, 2014). Numerous studies have demonstrated that germination improves texture and acceptability of BR and also enhances nutrient and phytochemical bioavailability (Komatsuzaki et al., 2005). During germination, significant changes in biochemical, nutritional and sensory characteristics occur resulting in the degradation of storage proteins and carbohydrates and promoting the synthesis and accumulation of biofunctional compounds. Germination process generally results in improved levels of vitamins, minerals, fibres and phytochemicals such as ferulic acid, GABA, γ-oryzanol and antioxidant activity (Cho and Lim, 2016).

Consumption of GBR is receiving increasing attention supported by scientific evidence on its beneficial health effects reducing the risk of diseases such as obesity (Lim et al., 2014), cardiovascular diseases (Imam et al., 2014; Mohd. Esa et al., 2011), type 2 diabetes (Imam and Ismail, 2013; Shen et al., 2015), neurodegenerative diseases (Azmi et al., 2013) and osteoporosis (Muhammad et al., 2013). In this context, GBR has been identified as a natural and inexpensive substitute of conventional white rice to improve nutritive and health status of a large world population (Wu et al., 2013).

Several studies have been carried out to optimize the germination conditions and maximize the beneficial attributes of GBR since the chemical composition of the grains change dramatically during germination (Cho and Lim, 2016). Lesser efforts, however,

have been dedicated to evaluate the effect of drying processes to preserve the quality and composition of the obtained GBR grains. Most of the research studies focused on the production and characterization of GBR use freeze-drying, process in which water is removed by sublimation producing high-value dried products with extended shelf-life (Karam et al., 2016). This technique maintains the color, shape, aroma and nutritional quality of the product and its relevance to preserve nutraceutical compounds has been highlighted (Argyropoulos et al., 2011). However, the process is slow and requires expensive equipment and, thus, it is rarely used for the preservation of foods on the industrial scale (Vega-Mercado et al., 2001). Drying operations as convective drying, hot-air oven, vacuum, osmotic, fluidized bed and superheated steam dehydration techniques are conventionally used to achieve water evaporation in shorter times. In GBR, drying procedure system and operation conditions affect the drying rate and quality attributes, whilst starch digestibility, GABA and dietary fiber content depend on the applied temperature (Chungcharoen et al., 2014; Srisang et al., 2011). These drying methods are still expensive and not always affordable in low and middle-income countries where rice production and transformation is performed with few economic resources.

Solar drying is the oldest preservation procedure for agri-food products and it is the most common method to dehydrate rice grains in those producers´ countries located in tropical areas of the world (Imoudu and Olufayo, 2000). Taking in mind its low energy cost, the aim of the present work was to evaluate for the first time the effect of sun-drying on the content of bioactive compounds in GBR. In this context, Ecuador is a tropical country which experiences little variation in daylight hours during the course of a year and temperatures oscillate between 30 and 40º C. These climate conditions could be favourable for the stabilization of GBR by sun-drying.

Our group has recently addressed different germination conditions to maximize the phytochemical content, antioxidant activity and nutritional features of three certified BR varieties and one experimental cultivar grown in Ecuador features (Cáceres et al., 2014a, b). In an effort to make an additional step towards sustainable and cost-effective production of Ecuadorian GBR, the present work was designed to assess the effect of different germination conditions on γ-oryzanol, GABA, total phenolic compounds and antioxidant activity in a highly produced rice variety, SLF09, and how sun-drying influence the content of those biologically active compounds. Dried GBR under sun might assure the intake of health-promoting compounds in that population where rice is the main food as ready-to-eat meals or soups after rehydration or to supplement functional foods as strategy for combating highly prevalent chronic diseases.

2. Material and methods

2.1. Rice samples

Commercial certified brown rice (BR) variety *indica* SLF09 was supplied by the company INDIA-PRONACA Co, Ecuador. This variety was selected based on its high harvest yield (6 Tm/Ha) and the consumer acceptability characterized by its translucent white center and extra-long shape grain.

2.2. Germination process

Fifty grams of BR were washed with distilled water and soaked in 0.1% sodium hypochloride (1:5; w/v) at 28 ºC for 30 min. After draining, BR grains were rinsed with distilled water to neutral pH. BR grains were then soaked in distilled water (1:5; w/v) at 28 ºC for 24 h. Afterwards, soaking solution was removed and the soaked BR grains were obtained.

Soaked BR were extended on drilled grilles over a moist laboratory paper and they were then covered with the same paper. The grille was placed in plastic germination trays containing distilled water in order to maintain the paper always wet by capillarity. Germination trays containing the soaked grains were introduced in a germination cabinet (model EC00-065, Snijders Scientific, Netherlands) provided with a circulating water system to keep the humidity > 90%. GBR were produced at 28 and 34 ºC in darkness for 48 and 96 h. Soaked and GBR grains were dehydrated in a freeze-drier (Freeze Mobile G, Virtis Company, INC Gardiner, NY, USA). Freeze-dried grains were finely ground in a ball mill (Glen Creston Ltd., Stanmore, UK), passed through a sieve of 0.5 mm and the obtained flour was stored under vacuum conditions in sealed plastic bags in darkness at 4 ºC until further analysis. Each germination process was carried out in triplicate.

2.3. Sun-drying proccess

Fresh soaked and GBR samples produced as explained above were lied out plastic cloths under sunlight in Guayaquil (Ecuador), at a latitude of 2º 12’ 21’’ S and a longitude of 79º 54’ 28’’ W, an elevation of 6 m above the sea level with temperatures between 30-40ºC (EXA, 2008) until GBR grains reached ~10% of moisture (Imoudu and Olufayo, 2000). Sun-dried soaked and GBR were finely ground in a ball mill (Glen Creston Ltd., Stanmore, UK), passed through a sieve of 0.5 mm and the flour obtained was stored under vacuum conditions in sealed plastic bags in darkness at 4 ºC until further analysis. Each drying process was conducted in triplicate.

2.4. Determination of moisture content

The content of moisture in dried soaked and GBR was determined by keeking the samples at 105 ºC to a constant weight according to AOAC 925.09 (AOAC, 2000).

2.5. Determination of γ-oryzanol.

The analysis of γ-oryzanol in rice samples was performed as previously reported (Cho et al., 2012) with some modifications. Briefly, 1 g of sample was mixed with 10 mL of 100% methanol and further sonicated for 10 min. The mixture was centrifuged at 15,000 rpm for 10 min at room temperature and then concentrated to dryness. Samples were then diluted in 1 mL of 100% methanol, filtered through a 0.45μm membrane and then analysed by HPLC. The HPLC system consisted of an Alliance Separation Module 2695 (Waters, Milford, USA), a photodiode array detector 2996 (Waters) setted at 325 nm wavelengh and Empower II software (Waters). Twenty microliters were injected onto a C18 column (150 x 3.9 mm i.d., 5 μm size, Waters). A gradient mobile phase was pumped at a flow of 1.0 mL/min to separate the γ-oryzanol components consisting in solvent A (acetonitrile), solvent B (methanol) and solvent C (bi-distilled water) for 50 min as follows: initial isocratic flow 60% solvent A, 35% solvent B and 5% solvent C for 5 min, gradient flow 60% solvent A and 40% solvent B for 3 min keeping it at isocratic flow for 2 min, then gradient flow 22% solvent A and 78% solvent B for 10 min, to be maintained isocratically for 15 min, and changing to initial conditions for 10 min and, finaly, isocratic conditions to equilibrate column for 10 min. γ-Oryzanol derivatives in rice samples were identified by retention time and spiking the sample with a commercial γ-oryzanol standard solution (Cymit, Spain) (Figure 1) and the purity of peaks was confirmed by spectra comparison and by MS analysis (Cho et al., 2012). Steryl ferulates components of γ-oryzanol were quantified by external calibration curves using γ-oryzanol standard solutions. Replicates samples were independently analyzed and results were expressed in mg γ-oryzanol/100 g of dry weight (d.w.).

2.6. Determination of γ-aminobutyric acid (GABA)

γ-Aminobutyric acid (GABA) content was determined by HPLC as described previously (Cáceres et al., 2014b). Briefly, 50 µL aliquot of concentrated water-soluble extract and 10µL allyl-L-glycine solution (Sigma-Aldrich) used as internal standard were derivatized with 30 µL phenyl isothiocyanate (PITC 99%, Sigma-Aldrich) and dissolved in mobile phase A for GABA analysis. An Alliance Separation Module 2695 (Waters, Milford, USA), a photodiode array detector 2996 (Waters) setted at 242 nm wavelenth and an Empower II chromatographic software (Waters) were used as chromatographic system. A volume of 20µL of sample were injected onto a C18 Alltima 250 x 4.6 mm i.d., 5 µm size (Alltech, Spain) column thermostatted at 30 ºC. The chromatogram was developed at a flow rate of 1.0 mL/min by eluting the sample with mobile phase A (0.1 M ammonium acetate pH 6.5) and mobile phase B (0.1 M ammonium acetate, acetonitrile, methanol, 44/46/10, v/v/v, pH 6.5). Replicates samples were independently analyzed and results were expressed as mg GABA/100 g d.w.

2.7. Determination of total phenolic content

The Folin-Ciocalteu's method was used for the quantification of total phenolic content (TPC), as previously reported (Cáceres et al., 2014b). The absorbance was measured at 739 nm using a microplate reader (Synergy HT, BioTek Instruments) and TPC were quantified by external calibration using gallic acid (Sigma-Aldrich) as standard. Sample replicates were independently analyzed and results were expressed as mg of gallic acid equivalents (GAE)/100 g d.w.

2.8. Determination of antioxidant activity

Antioxidant activity was determined by the method of oxygen radical absorbance capacity (ORAC) by fluorescence detection (λ_{exc} 485 nm and λ_{em} 520 nm) using an automatic plate reader (BioTek Instruments), previously described (Cáceres et al.,

2014b). Sample replicates were independently analyzed and results were expressed as mg of Trolox equivalents (TE)/100g of sample d.w.

2.9. Statistical analysis

Each germination experiment and subsequent drying process were conducted in triplicate. Two extractions were performed for each replicate and the analytical determinations were carried out in triplicate. Data were expressed as mean ± standard deviation. The data obtained from each experimental condition were subjected to one-way analysis of variance (ANOVA) using Duncan test to determine the significant differences at $P \leq 0.05$ level using Statgraphics Centurion XVI Program, version 16.1.17 (Statistical Graphics Corporation, Rockville, Md) for Windows. This programme was also used for correlation analysis.

3. Results

In order to study the effect of germination on biologically active compounds of BR, soaked and GBR were freeze-dried as this drying process minimize its degradation and deterioration In parallel, fresh soaked and GBR were dried under the sun and the moisture content ranged between 9.5-12.5

3.1. Effect of germination on γ-oryzanol content in brown rice variety SLF09

BR variety SLF09 exhibited four main chromatographic peaks that unambiguously were identified as cycloartenyl ferulate (peak 1), 24-methylene cycloartanyl ferulate (peak 2), campestryl ferulate (peak 3) and sitosteryl ferulate (peak 4) (Figure 1), confirmed by spicking with commercial standard γ-oryzanol by HPLC and mass espectrometry analysis. The quantitative results revealed that 24-methylene cycloartanyl ferulate (peak 2) was present in the larger amount (4.98 mg/100g d.w.),

followed by cycloartenyl ferulate (peak 1) and campestryl ferulate (peak 3) (2.6 and 2.24 mg/100g d.w., respectively) and, finally, sitosteryl ferulate (peak 4) (1.34 mg/100g d.w.), accounting for a total amount of 11.17 mg γ-oryzanol/100g d.w. (Table 1). Total content of γ-oryzanol underwent a significantly decrease ($P \leq 0.05$) during the initial soaking treatment (from 11.17 to 9.23 mg/100g d.w.) and a 17% reduction was observed. This effect was due to drops exhibited by the individual derivatives: Campestryl ferulate suffered the largest decrease (25%), followed by sitosteryl ferulate (20%) and, in less amount, cycloartenyl and 24-methylene cycloartanyl ferulates (15%) (Table 1). Germination process did not bring about further γ-oryzanol losses, since most of the steryl derivative concentrations kept almost unchanged ($P \geq 0.05$), and concentrations ranged from 9.2 to 9.64 mg/100g d.w. in GBR grains (Table 1).

In an attempt to stablish the proportion of each individual derivative within the total γ-oryzanol content before and after germination, the contribution of each steryl ferulate to the total γ-oryzanol content was calculated (Figure 2). In crude BR, 24-methylene cycloartanyl ferulate was the predominant one (45%), followed by cycloartenyl ferulate (23%), then campestryl ferulate (20%) and, finaly, sitosteryl ferulate (12%). These proportions were mainteined almost invaried after soaking and slight modifications were appreciated in GBR samples. While the contributions of cycloartenyl and sitosteryl ferulates did not change during germination, those for 24-methylene cycloartanyl and campestryl ferulates were modified to aproximately 48 and 17%, respectively (Figure 2).

3.2. Effect of germination on GABA content in brown rice variety SLF09

Table 2 reports the GABA content in ungerminated, soaked and GBR. Variety SLF09 showed a concentration of 1.07 mg GABA/100g d.w. that increased 7-fold after

soaking process carried out at 28 ºC for 24 h. During germination, a gradual and time-dependent accumulation of GABA was achieved and 28 ºC produced larger amounts of this compound (34.8 mg/100 g d.w. and 99 mg/100g d.w. for 48 and 96 h, respectively) than 34 ºC (24.3 mg/100g d.w and 83.6 mg/100g d.w. for 48 and 96 h, respectively).

3.3. Effect of germination on the content of total phenolic compounds in brown rice variety SLF09

Changes in total phenolic compounds (TPC) of BR at different germination conditions are presented in Table 2. The TPC in crude samples corresponded to 132.53 mg GAE/100g d.w. and this content underwent a significantly ($P \leq 0.05$) decrease after steeping process (113.23 mg GAE/100g d.w.). Germination, however, led to a sharp increment in the concentration of these compounds with time, reaching values of 187.17 and 176.48 mg GAE/100g d.w. for 48h-GBR and of 298.23 and 382.99 mg GAE/100g d.w. for 96h-GBR, at 28 and 34 ºC, respectively.

3.4. Effect of germination on the antioxidant activity in brown rice variety SLF09

The total antioxidant activity of crude, soaked and GBR grains determined by the ORAC-FL method is also collected in Table 2. The antioxidant activity of non-germinated SLF09 grains was 494.81 mg TE/100g d.w. and soaking did not cause significant ($P \geq 0.05$) changes. During germination process, the antioxidant activity increased gradually following a time-dependent pattern and higher temperature led to higher levels. Thus 48h-GBR samples exhibited 554.85 and 662.8 mg TE/100g d.w. at 28 and 34 ºC, respectively, whilst 96h-GBR grains showed larger activity (977.47 and 1079.35 mg TE/100g d.w. for those respective temperatures). However, there was not

found a significant correlation between antioxidant activity and γ-oryzanol content of GBR (freeze-dried) samples (Figure 6C).

3.5. Effect of sun-drying on the content of γ-oryzanol, GABA, TPC and antioxidant activity of germinated brown rice variety SLF09

Tables 1 and 2 include the content of γ-oryzanol, GABA, TPC and antioxidant activity in sundried soaked and GBR. This drying process increased the content of γ-oryzanol in GBR from 14.1 mg/100g d.w. in soaked and 28 ºC/48h-GBR samples to 18.2 mg/100g d.w. in 28 ºC/96h-GBR ones, representing a 34 and 48 % increment, respectively. Sundried 34 ºC/48h-GBR and 34 ºC/96h-GBR showed γ-oryzanol concentrations of 16.7 mg/100g d.w., accounting for an increment of 42% (Figure 3). These amounts are the result of the accumulation of the individual steryl ferulates during sun-drying that reached values in the range of 2.6-3.56 mg/100g d.w. for cycloartenyl ferulate, 6.07-7.7 mg/100g d.w. for 24-methylene cycloartanyl ferulate, 3.56-4.64 mg/100g d.w. for campestryl ferulate and 1.78- 2.30 mg/100g d.w. for sitosteryl ferulate (Table 1). Figure 2 illustrates the contributions of individual steryl ferulates to the total γ-oryzanol content. Sun-drying increased the proportion of campestryl ferulate to approximately 25-26%, whilst cycloartenyl ferulate and 24-methylene cycloartanyl ferulate decreased to 18-19% and 42-43%, respectively, whilst sitosteryl ferulate was not modified.

The content of GABA in sundried GBR grains is found in Table 2. The largest GABA accumulation was achieved for those samples previously germinated for 96 h (49.8 and 66.4 mg/100g d.w. at 28 and 34 ºC, respectively), whilst temperature did not modified GABA content in GBR for 48 h (~36.5 mg/100g d.w.) and soaked BR provided the lowest GABA content. Sun-drying only increased GABA content in

soaked and 34 ºC/48h GBR (41 and 33%, respectively), did not cause significant GABA modification in 28 ºC/48h GBR, while for those BR grains germinated for 96h, sun-drying led to unexpected GABA losses (99 and 24% at 28 and 34ºC, respectively) (Figure 3).

Sun-drying brought about slight changes in TPC content of GBR and only in those germinated for 96 h, sun-drying led to significant ($P \leq 0.05$) enhancement of TPC (Table 2, Figure 3). However, the antioxidant activity underwent a gradual and significant ($P \leq 0.05$) increase in sundried GBR that was higher for those GBR produced at 28 ºC (978.6 and 1283.25 mg TE/100 g d.w. for 48 and 96 h, respectively), althought those germinated at 34 ºC also provided a large ORAC value (826.8 and 1174.9 mg TE/100 g d.w. for 48 and 96 h, respectively). In all the samples, sun-drying caused a sharp increment in antioxidant activity compared with the GBR counterparts (Figure 3).

In an attempt to elucidate the potential compounds responsible for antioxidant activity, Figure 4shows the correlation between ORAC values and TPC and γ-oryzanol content in GBR and sundried GBR. A significant positive correlation ($P \leq 0.05$) was found between ORAC and γ-oryzanol (Figure 4A) ($r=0.82$) and TPC (Figure 4B) ($r=0.86$) of sundried GBR, and between antioxidant activity and TPC content of GBR (Figure 4D) ($r=0.96$).

4. Discussion

BR variety SLF09 is largely produced in Eduador by INDIA-PRONACA and exported to other Latin American countries. It is one of the long grain rice *indica* varieties highly consumed due to this varity of rice remains loose after cooking. In Ecuador, this rice is produced at local farmlands that currently reach overproduction (Cáceres et al., 2014a), mainly used for animal feeding and, hence, undervaluaded.

Therefore, germination of BR emerges as a simple cost-effective strategy for enhancing the content of bioactive compounds. In addition, economic, effective and sustainable sun-drying provided by Ecuadorian climatology provided moisture content lower than 15 %, in acordance to Imoudu and Olufayo (2000), contributing to the preservation of GBR for further storage, comercialization and consumption as ready-to-eat staple food or to be incorporated in demanded functional foods with added-value (Cornejo et al., 2015). In this context, GBR can contribute to reduce the risk of cardiometabolic diseases in those populations where rice constitute the main energy and nutrient food without altering the existing consumption habits (Ochoa-Avilés et al., 2014).

The composition of GBR depends on many factors such as genotype diversity, soaking conditions, germination time and temperature, as well as drying process. It is well known that germination process generally improves the nutritional quality, by augmenting the protein digestibility, vitamins, minerals and inducing the formation of bioactive components (Cho and Lim, 2016).

In our study, BR variery SLF09 provides γ-oryzanol in the form of four main derivatives. A wide range of variation for total γ-oryzanol has been reported previously in varieties of BR from different geographical origin (Cho et al., 2012; Khatoon and Gopalakrishna, 2004; Kiing et al., 2009; Miller and Engel, 2006; Ohtsubo et al., 2005; Pereira-Caro et al., 2013). Values ranging from 1.2 mg/100g in BR varieties from the Camargue region of France (Pereira-Caro et al., 2013) to 313 mg/100g in a BR cultivar cultivated in Taiwan (Huang and Ng, 2012) have been reported. The amounts of γ-oryzanol found in BR variety SLF09 is comparable to those previously reported in three *indica* cultivars grown in Brazil (Pascual et al., 2013), and in eight cultivars from South Sarawak, Malaysia (Kiing et al., 2009). The contribution of each steryl ferulate to total γ-oryzanol content lies within the range previously reported in different French rice

varieties (Pereira-Caro et al., 2013) and differ to those observed in long BR grain cultivars (Miller and Engel, 2006), in which the largest proportion was accounted by cycloartenyl ferulate (43-48%), followed by 24-methylene cycloartanyl ferulate (26-29%) and, in minor proportions, campestryl ferulate (17-21%) and sitosteryl ferulate (7-8%). The different proportions of individual γ-oryzanol constituents have been attributed to the variability among genotypes.

During germination process, γ-oryzanol underwent a significant decrease (15 %) that occurred mainly during the initial hydration process and not further changes during germination were found. Results from the literature about the effect of germination on the content of total γ-oryzanol in BR are not coincident possibly due to the different germination conditions used. Our results are in accordance with those previously reported in several BR cultivars from Malaysia (Kiing et al., 2009) where a decrease of γ-oryzanol after germination at 25 ºC for 24 h was observed, and differ to Thai cultivar RD-6 that underwent an increase after 12 h-soaking and further 24 h-germination at 28-30 ºC (Moongngarm and Saetung, 2010), or to those Indian varieties IR 64 and BPT that did not show changes in γ-oryzanol content after 16 h of soaking followed by120 h of germination at room temperature (Jayadeep and Malleshi, 2011). During the germination process hydrolytic enzymes are activated and the effect observed on γ-oryzanol could be due to the induction of feruloyl esterases activity during the initial soaking process (Sancho et al., 1999). In addition, steryl ferulate degradation was also attributed to dynamic ferulic acid metabolism during BR hydration (Tian et al., 2004). Nevertheless, our results indicate that individual steryl ferulate contribution remained almost constant throughtout germination process showing that these compounds seems to be stable after 96 h at 28 and 34 ºC, effect that has not been reported previously.

GBR were sundried and γ-oryzanol increased between 34 and 48%, results reported for the first time in this work. These outcomes evidence the accumulation of γ-oryzanol derivatives during drying under solar exposition that can be attributed to the sunlight effect on ferulic acid metabolism and further synthesis of individual γ-oryzanol components. It has been reported that sunlight has a profound effect on the biosynthesis of ferulic acid esters by affecting the metabolic activation of enzymes involved in the defence mechanism to radiation (Wang et al., 2014), and also in the development of new plant structural tissues (Hoson and Wakabayashi, 2015). To our knowledge, this is the first report describing the effect of sun-drying on γ-oryzanol content and composition evidencing GBR as a rich source of γ-oryzanol.

It is widely recognized that γ-oryzanol and its individual components are natural antioxidant. Among them, 24-methylene cycloartenyl ferulate exhibited the greatest antioxidant potential (Xu et al., 2001) and, together with cycloartenyl ferulate, showed anti-inflammatory properties (Akihisa et al., 2000). In addition, γ-oryzanol has shown anti-atherogenic, anti-cholesterolemic, hypolipidemic and anti-cancer effects *in vivo* (Wilson et al., 2007). γ-Oryzanol is administrated to the treatment of diabetes, menopause, allergies and gastrointestinal inflammatory diseases (Lemus et al., 2014). These properties make γ-oryzanol one of the most demanding compounds for nutraceutical, pharmaceutical and cosmeceutical preparations (Ghatak and Panchal, 2011). Our results show that sun-drying may improveeven more γ-oryzanol content in GBR, and can be considered as a sustainable bio-efficient process to develop γ-oryzanol enriched GBR.

GABA is usually present as a minor compound in crude grains, however, germination boosts its accumulation in rice sprouts (Cáceres et al., 2014b; Ohtsubo et al., 2005). GABA synthesis is usually initiated as consequence of the activation of

glutamate decarboxylase (GAD) enzyme during soaking process, activity that increases with germination time whilst temperature seems to exert a minor impact (Cáceres et al., 2014b; Roohinejad et al., 2011). GAD catalyses the decarboxylation of glutamic acid to GABA and CO_2 and it has been established a range between 20 and 40 ºC as optimal temperature for enzyme activity (Yang et al., 2013). Additionally, GABA can also be synthetized from putrescine as a response to abiotic stress during germination (Shelp et al., 2012) contributing to the overall account of GABA in GBR. Our results are consistent with those published recently for GBR, with values ranging from 34.5 to 140 mg GABA/100g d.w. (Cáceres et al., 2014b; Hayat et al., 2015; Roohinejad et al., 2011; Yang et al., 2013; Zhang et al., 2014).

The drying process of GBR under sunlight had a different effect on GABA depending on germination conditions and higher amounts were only found in soaked BR and 34 ºC/48h GBR. These results can be partly attributable to some remaining GAD activity after germination due to the activity of this enzyme at temperatures below 40 °C (Kim et al., 2014). GABA diminution was observed in those dried samples previously germinated for 96h, results that could be attributed to sunlight exposure activation of GABA shunt pathway. These metabolic pathway uses GABA as precursor for the synthesis of succinic acid required in the Krebs cycle (Fait et al., 2008). Nevertheless, the content of GABA in sundried GBR has been described for the first time in the present work, ranging from 12 mg/100g in soaked grains to 67 mg/100g in 34 ºC/96h GBR. GABA has a well-known antihypertensive and it has been reported that a daily GABA intake of 20 mg caused a reduction of blood pressure in individuals with pre-hypertension (Inoue et al., 2003). Furthermore, a daily dose of 26.4 mg of GABA seems to be effective in the treatment of neurological disorders (Diana et al., 2014).

Taking into account that 100 g of sun-dried GBR provide between 1.5 to 3-fold these required amounts, its consumption would provide health beneficial effects.

BR is considered a good source of phenolic compounds and the content in the variety SLF09 is within the range previously reported (Ti et al., 2014). TPC content increased sharply as consequence of germination time while temperature had a minor influence (Cáceres et al., 2014b). This increment was partially explained by the production of enzymes that hydrolyse fiber components during GBR germination (Tian et al., 2004). In addition, the action of endogenous esterases can release free phenolics required for the synthesis of more complex compounds (Hatfield et al., 1999) providing, at the same time, defence against environmental agents (Lemus et al., 2014). Moreover, germination induces the expression of phenylalanine ammonia-lyase producing cinnamic acid from phenylanine which is, then, metabolized into other free phenolic acids (Shih et al., 2008). GBR obtained at 34ºC for 96 h in the present work exhibited greater TPC content than those reported previously (Cáceres et al., 2014b; Moongngarm and Saetung, 2010; Ti et al., 2014). Ti et al., (2014) identified protocatechuic, chorogenic, caffeic and ferulic acids as the main phenolic acids and the later was the most abundant (357 µg/g d.w. after 5 day-germination).

Sun-drying kept or, even, increased the content of TPC (Figure 3) although a drop due to their susceptibility to oxidation during light exposure should be expected. TPC increase after sun-drying of GBR could be explained by activation of the phenylpropanoid pathway that occur in response to environmental factors (Reilly et al., 2014; Shih et al., 2008) and increased exposure to UV-B light (Du et al., 2014). To our knowledge, we present here inedited results describing the sun-drying effect on the content of TPC of GBR that provide 176 to 383 mg GAE/100g d.w. depending on previous germination conditions. Phenolic compounds are considered bioactive

compounds with health implications (Roleira et al., 2015). Particularly, ferulic acid bound to dietary fiber plays an important role in the prevention of colon cancer and attenuates inflammation (Shao and Bao, 2015). Moreover, soluble phenolic acids inhibit the oxidation of LDL cholesterol and the cell membrane liposomes enhancing mental health, immunity and protecting against diabetes deterioration (Chandrasekara and Shahidi, 2011). Therefore, sundried GBR can be considered an important source of phenolic compounds with beneficial attributes.

The antioxidant activity found in BR was higher than those observed in different Ecuadorian BR (Cáceres et al., 2014b), ranging between 242.7 and 316.8 mg TE/100g d.w., and differ to those reported by (Ti et al., 2014), who found levels of 38.7 µmol TE/g in BR variety Tianyou 998. This variability on antioxidant activity in crude grains could be attributed to the phenolic composition in different BR genotypes as well as to the contribution of other antioxidant compounds such as γ-oryzanol and vitamin E isomers (Cáceres et al., 2014b; Moongngarm and Saetung, 2010). Germination enhanced the antioxidant potential of BR variety SLF09, in agreement with previous studies (Cáceres et al., 2014b; Ti et al., 2014; Tian et al., 2004). During germination of BR, antioxidant activity was time and temperature dependent, as recently reported (Cáceres et al., 2014b), most likely caused by the accumulation of compounds with peroxyl-scavenging activity such as phenolic compounds (Andriantsitohaina et al., 2012; Zhou et al., 2014), as it was confirmed by the positive correlation obtained between antioxidant activity and TPC (Figure 4D). In addition, it has been suggested that soluble phenolic compounds account for 30% of the antioxidant activity (Adom and Liu, 2002). Nevertheless, γ-oryzanol can also contribute to the overall antioxidant activity (Aguilar-Garcia et al., 2007), however, in the present study, positive correlation between them in GBR was not found (Figure 4C). Other antioxidant compounds such as

tocopherols, tocotrienols, phytates and vitamin C could also contribute to this biological activity (Fardet, 2010; Frias et al., 2005). In sundried GBR samples, antioxidant activity was always significantly ($P \leq 0.05$) higher than their germinated counterparts, phenomenon that can be attributed to the increase observed in bioactive compounds such as γ-oryzanol and polyphenols. This hypothesis was confirmed by the positive correlations found between them (Figure 4A and 4B, respectively). Recent research shows that antioxidant activity of GBR is associated with the prevention of oxidative stress-related diseases (Lemus et al., 2014). It has been reported that GBR increases antioxidant enzyme activity and reduces lipid peroxidation in hypercholesterolimic rabbits (Mohd. Esa et al., 2011). To our knowledge, this is the first study showing antioxidant activity of sun-dried GBR and its consumption could contribute to ameliorate oxidative stress-induced diseases.

4. Conclusions

Germination conditions modify the content of biologically active compounds of BR variety SLF09. γ-Oryzanol decreased slightly during germination and sun-drying led to an important accumulation. GABA was synthetized during germination in a time-dependent manner and underwent significant rises after sun-drying only in those germinated for 48 h. TPC and antioxidant activity increased during germination that were preserved or even enhanced under solar dehydration. These outcomes show germination as a simple and sustainable process to preserve BR bioactive compounds and reveal, for the first time, the effectiveness of sun-drying for maximizing their accumulation. The obtained sun-dried GBR can be consumed directly after rehydatation as staple food or, after a milling process, can be incorporated in bakery products and pasta (Cornejo et al., 2015). In this context, consumption of sundried GBR can take

place as parbolished rice to feed children and adolescents contributing to the control of metabolic related disorders (Ochoa-Avilés et al., 2012).

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Table 1. Content of γ-oryzanol components (mg/100g dw) in crude, soaked and germinated brown rice variety SFL09 and the effect of sun-drying.

BR samples	Cycloartenyl ferulate (Peak 1)	24-Methylene cycloartanyl ferulate (Peak 2)	Campestryl ferulate (Peak 3)	Sitosteryl ferulate (Peak 4)	Total γ-oryzanol
Crude	2.60±0.05^{b}	4.98±0.07^{d}	2.24±0.03^{bA}	1.34±0.02^{b}	11.17±0.10^{b}
Freeze-dried					
Soaked 28ºC, 24h	2.21±0.04^{a}	4.27±0.06^{aA}	1.67±0.05^{aA}	1.08±0.02^{aA}	9.23±0.08^{aA}
Germinated					
28ºC, 48h	2.22±0.06^{aA}	4.32±0.12^{abA}	1.61±0.05^{aA}	1.05±0.03^{aA}	9.20±0.20^{aA}
28ºC, 96h	2.32±0.07^{aA}	4.52±0.10^{bcA}	1.58±0.03^{aA}	1.09±0.02^{aA}	9.52±0.17^{aA}
34ºC, 48h	2.33±0.13^{aA}	4.56±0.20^{cA}	1.59±0.15^{aA}	1.11±0.08^{aA}	9.59±0.56^{aA}
34ºC, 96h	2.36±0.11^{aA}	4.58±0.19^{cA}	1.60±0.09^{aA}	1.10±0.05^{aA}	9.64±0.42^{aA}
Sun-dried					
Soaked 28ºC, 24h	2.63±0.11^{b}	6.07±0.18^{eB}	3.56±0.13^{cB}	1.82±0.03^{bB}	14.08±0.19^{bB}
Germinated					
28ºC, 48h	2.60±0.05^{bB}	6.08±0.17^{eB}	3.65±0.09^{cB}	1.78±0.03^{bB}	14.09±0.21^{bB}
28ºC, 96h	3.56±0.05^{cB}	7.70±0.09^{fB}	4.64±0.09^{eB}	2.30±0.03^{cB}	18.18±0.17^{dB}
34ºC, 48h	3.24±0.06^{cB}	7.23±0.04^{fB}	4.15±0.04^{dB}	2.11±0.01^{cB}	16.75±0.09^{cB}
34ºC, 96h	3.09±0.12^{cB}	7.21±0.11^{fB}	4.36±0.15^{eB}	2.07±0.02^{cB}	16.73±0.07^{cB}

Data are the mean values ± standard deviation of three independent experiments (n=3). Lowercase letters indicate statistical differences among germination conditions ($P \leq 0.05$ according to Duncan's test). Uppercase letters indicate statistical differences among drying process for a same germination conditions ($P \leq 0.05$ according to Duncan's test).

Table 2. Content of γ-aminobutyric acid (GABA), total phenolic compounds (TPC) and antioxidant activity (ORAC) of crude, soaked and germinated brown rice and the effect of sun-drying**.**

BR samples	GABA (mg/100g)	TPC (mg GAE/100g dm)	ORAC (mg TE/100g dm)
Crude	1.07 ± 0.09^{a}	132.53 ± 2.78^{b}	494.81 ± 19.71^{a}
Freeze-dried			
Soaked 28ºC, 24h	7.46 ± 0.12^{bA}	113.23 ± 7.77^{aA}	508.41 ± 12.49^{abA}
Germinated			
28ºC, 48h	34.84 ± 2.78^{dA}	187.17 ± 3.19^{dA}	554.85 ± 17.59^{bA}
28ºC, 96h	99.03 ± 4.83^{fA}	298.23 ± 13.48^{eA}	977.47 ± 62.49^{dA}
34ºC, 48h	24.33 ± 0.44^{cA}	176.48 ± 3.02^{cA}	622.80 ± 18.60^{cA}
34ºC, 96h	83.60 ± 2.67^{eA}	382.99 ± 10.44^{gA}	1079.35 ± 69.70^{dA}
Sun-dried			
Soaked 28ºC, 24h	12.75 ± 0.50^{gB}	118.14 ± 5.30^{fA}	547.66 ± 25.22^{eA}
Germinated			
28ºC, 48h	36.41 ± 2.67^{hA}	190.29 ± 8.55^{gA}	978.63 ± 30.33^{fB}
28ºC, 96h	49.85 ± 4.62^{iB}	359.22 ± 12.35^{hB}	1283.25 ± 74.04^{iB}
34ºC, 48h	36.50 ± 1.36^{hB}	195.13 ± 18.26^{gA}	826.82 ± 54.82^{gB}
34ºC, 96h	66.94 ± 1.21^{jB}	429.34 ± 17.54^{iB}	1174.88 ± 45.48^{hA}

Data are the mean values ± standard deviation of three independent experiments (n=3). Lowercase letters indicate statistical differences among germination conditions ($P\leq0.05$ according to Duncan's test). Uppercase letters indicate statistical differences among drying process for a same germination conditions ($P\leq0.05$ according to Duncan's test).

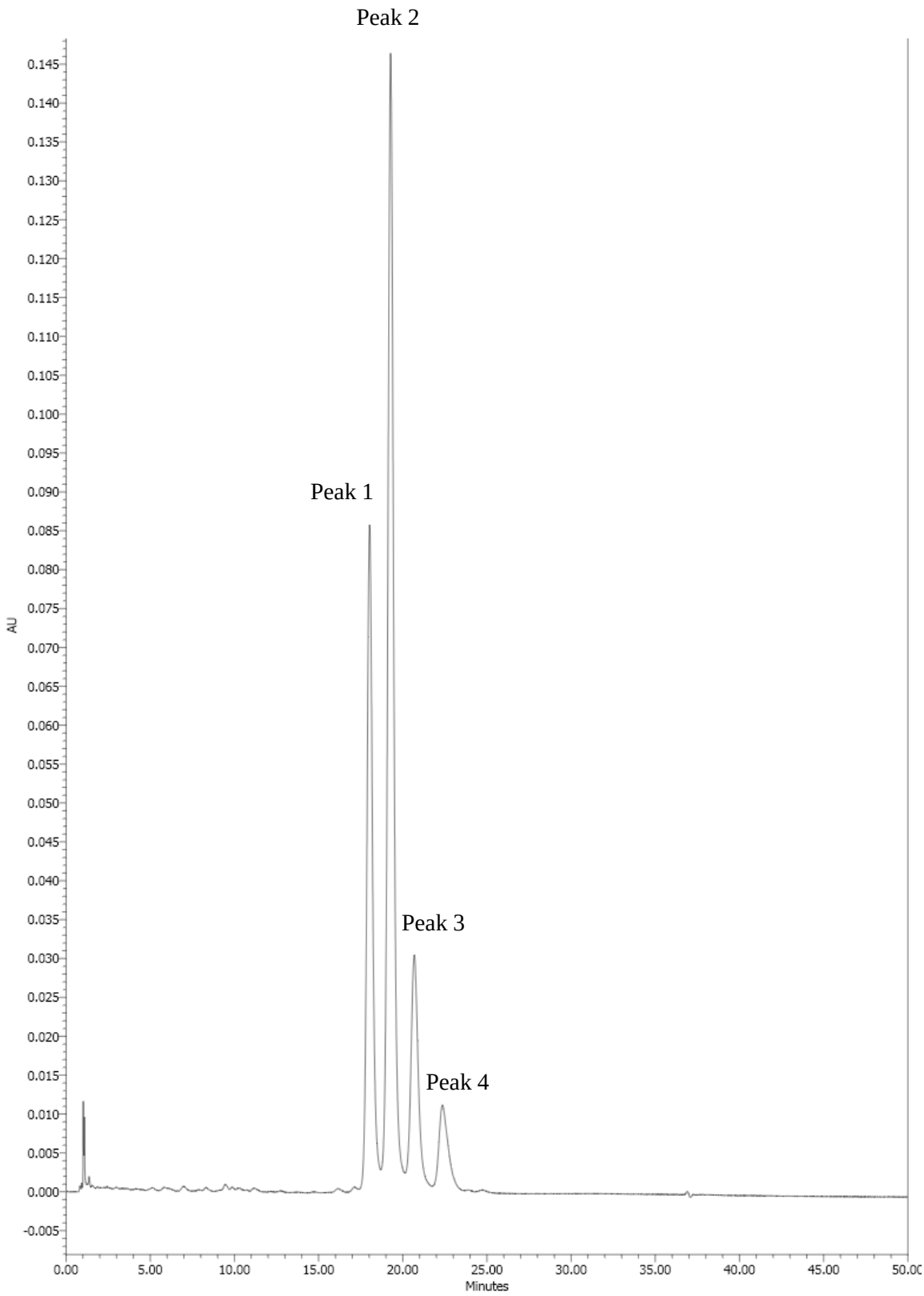

Figure 1. Chromatogram of γ-oryzanol standard. Peak 1, cycloartenyl ferulate; peak 2, 24-methylenecycloartanyl ferulate; peak 3, campesteryl ferulate; peak 4, sitosteryl ferulate.

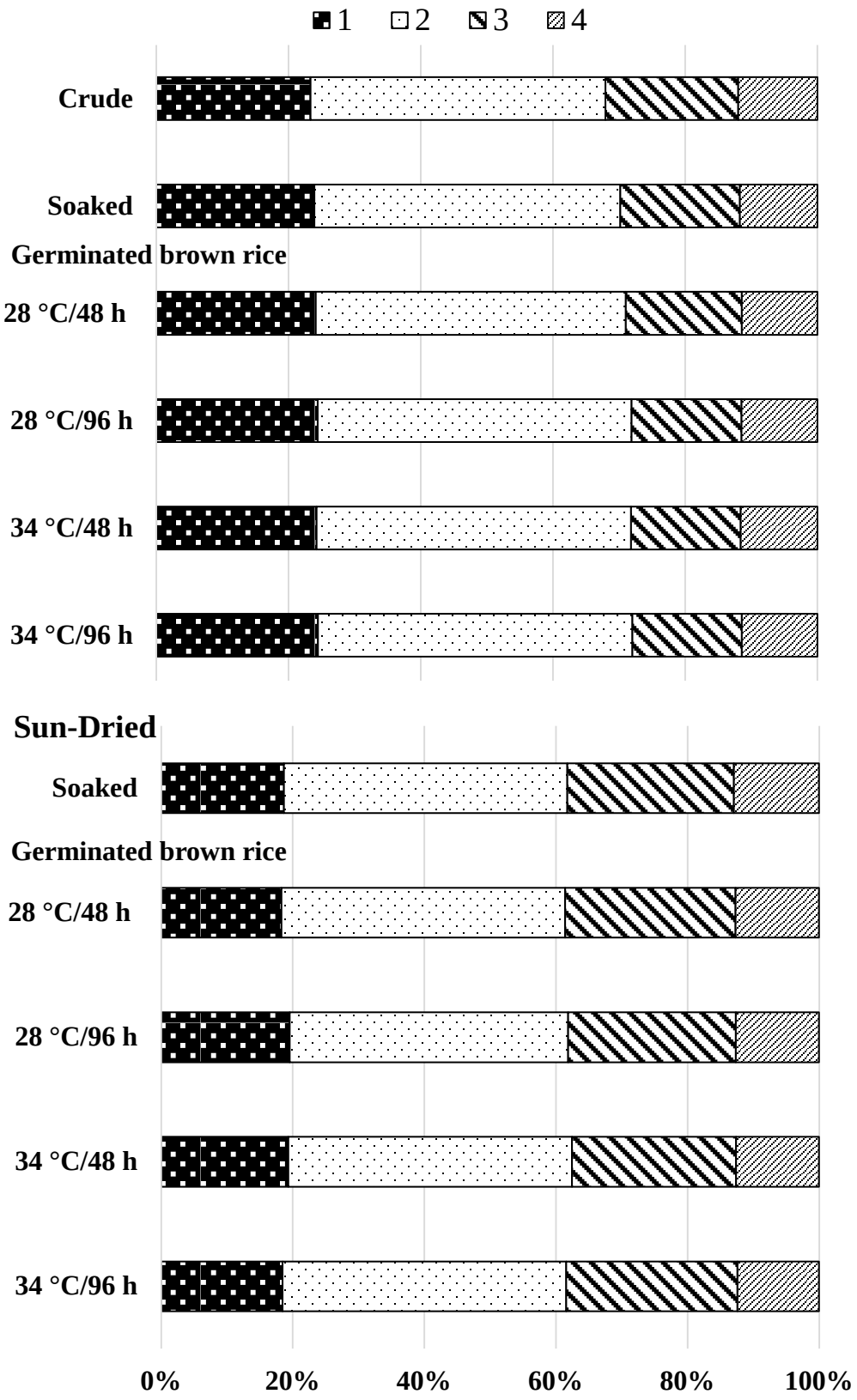

Figure 2. Contribution of the individual steryl ferulates to total content of γ-oryzanol in crude, soaked, and germinated brown rice and effect of sun-drying. 1, cycloartenyl ferulate; 2, 24-methylenecycloartanyl ferulate; 3, campesteryl ferulate; 4, sitosteryl ferulate.

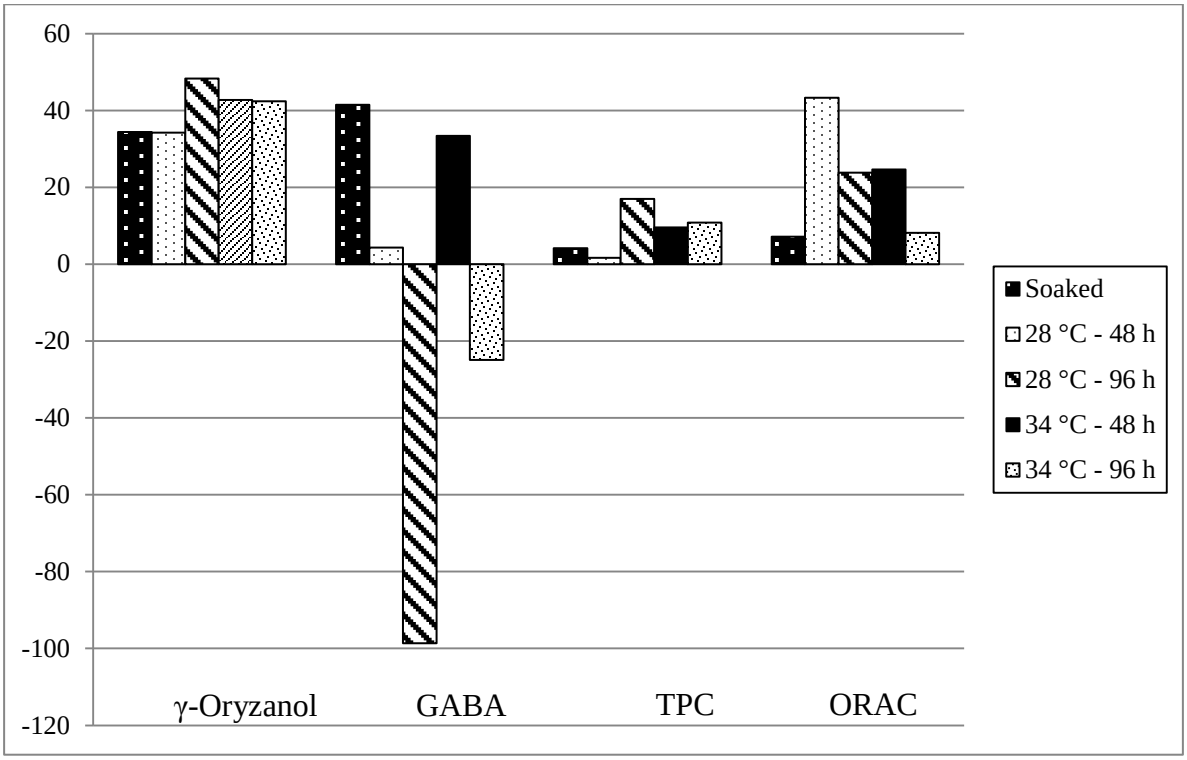

Figure 3. Effect of sun-drying on bioactive compounds and antioxidant activity of soaked and germinated brown rice, indicating increase percentages (positive y-axe) or decrease percentages (negative y-axe).

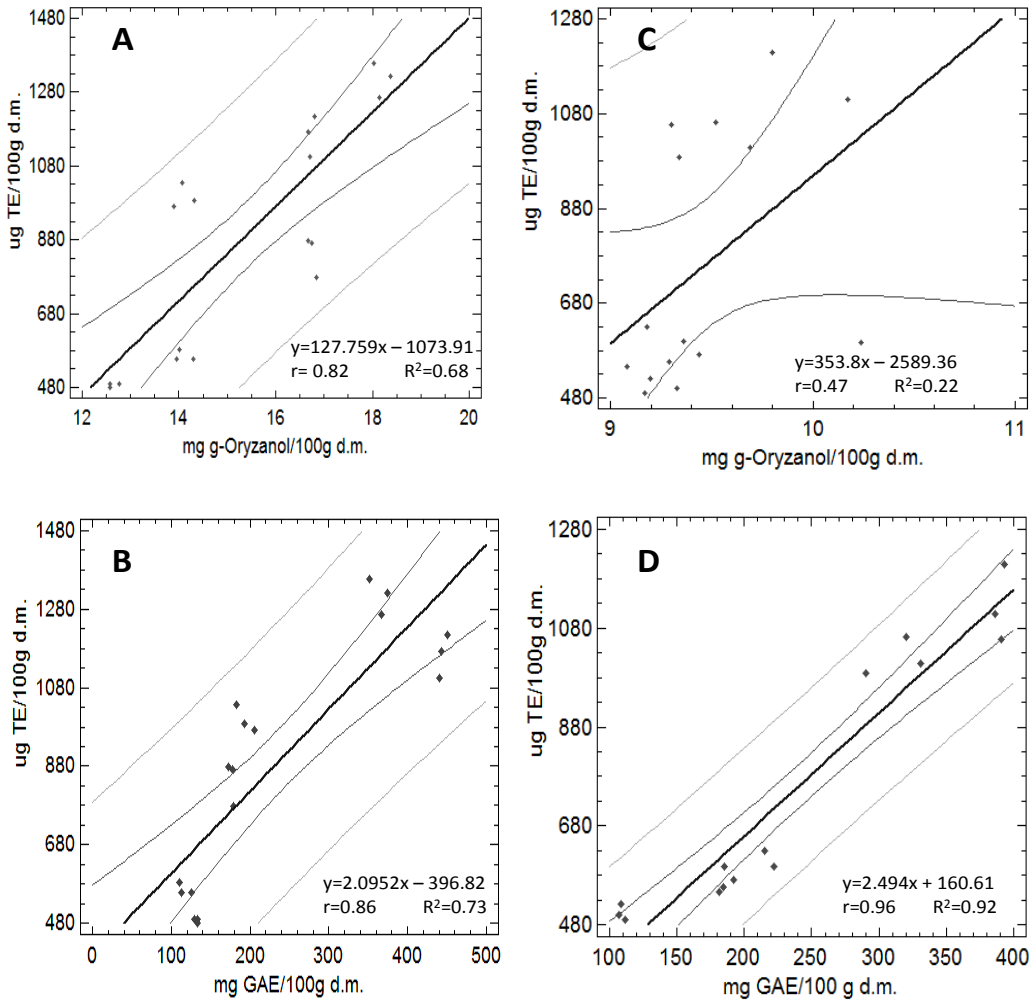

Figure 4. Antioxidant activity correlated (r) with the content of γ-oryzanol (A) and TPC (B) of SD-BR germinated and with the content of γ-oryzanol (C) and TPC (D) of FD-BR germinated. R^2 indicates the percentage of variation explained by the model.