Assessment on proximate composition, dietary fiber, phytic acid and protein hydrolysis of germinated Ecuatorian brown rice

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Abstract (150-250)

Germinated brown rice (GBR) is considered healthier than brown rice (BR) but its nutritive value has been hardly studied. Since nutritive quality of GBR depends on genetic diversity and germination conditions, six Ecuadorian BR varieties were germinated at 28 and 34 ºC for 48 and 96 h in darkness and proximate composition, dietary fiber fractions, phytic acid content as well as degree of protein hydrolysis and peptide content were studied. Protein, lipids, ash and available carbohydrate ranged 7.3-10.4%, 2.0-4.0%, 0.8-1.5% and 71.6 to 84.0%, respectively, in GBR seedlings. Total dietary fiber increased during germination (6.1-13.6%), with a large proportion of insoluble fraction, while phytic acid was reduced noticeably. In general, protein hydrolysis occurred during germination was more accused at 28 ºC for 48 h. These results suggest that GBR can be consumed directly as nutritive staple food for a large population worldwide contributing to their nutritional requirements.

Keywords: Brown rice, germination, proximate composition, dietary fiber, phytic acid, protein hydrolysis.

Introduction

Rice (*Oryza sativa* L.) is the most important staple food in many Asian and South American countries, but its consumption is widespread all over the world providing the energy and proteins over half the world´s population. Rice production has grown steadily in recent years, expecting to achieve a world production of 512 million tons by 2016 [1]. In Ecuador, rice is the main energy provider since it is the primary component of the main day course. Different varieties of long grain rice supplied by the Ecuadorian government and private enterprises are provided and farmland has recently met local demand to overproduction [2]. BR contributes not only with the basic nutrients of polished rice but also with bioactive compounds and antioxidants that are concentrated in the bran, being particularly rich in linoleic acid, γ-aminobutyric acid, tocopherols, tocotrienols, γ-oryzanol and phenolic compounds, as well as functional proteins, unsaponifiables lipids and dietary fiber that make BR attractive beyond its nutritional quality [3].

The interest of soaking and germination of BR is enormously increasing nowadays, since it is being demonstrated that the health benefits of BR are enhanced with germination [4]. It has been recently shown that germinated brown rice (GBR) exerts chemopreventive and immunomodulatory activity [5], suppresses inflammatory responses [6], inhibits adipogenesis [7] and attenuates hydrogen peroxide-induced oxidative stress [8].

Germination starts with a short soaking or steeping process in water where grain rouse from dormancy. During water uptake, the dry seeds restore their metabolic activities leading to biochemical, nutritional and sensorial changes. Seed germination is promoted by the regulation of different proteins involved in storage reserve degradation, biosynthesis of germination-promoting hormones, detoxification and defense and reinforcement of cell walls [9]. Germination results in increased reducing sugars, reduced amylose and starch granules became smaller and less homogeneous [10]. Grain storage proteins are partially hydrolyzed to peptides and amino acids improving protein digestibility and technofunctional properties [11]. Soaking and germination process lead to reduced phytic acid content [12] that results in a higher mineral bioaccesibility [13,14]. Likewise, dietary fiber (DF) content increases [15] and the substantial higher content of insoluble fiber can provide potential benefits in the prevention of diabetic vascular complications [16].

Recent studies have shown the bioactive compound enhancement during BR germination [14,17] and its effect on human health [4]. However, to our knowledge, there are no systematic studies addressing the effect of different germination conditions on proximate composition and related nutritive attributes in different BR varieties. Therefore, the objective of this work was to evaluate the effect of different germination conditions on proximate composition, dietary fiber and phytic acid content, as well as the protein hydrolysis and peptide content in different Ecuadorian BR varieties. The results will identify germination conditions with higher nutritive potential and will contribute to expand food compositional database showing GBR as an excellent nutritive food for improving the health status of the population worldwide.

Material and Methods

Plant materials

Experimental BR cultivar GO39839 (coded GO) and three commercial varieties INIAP 14, INIAP 15 and INIAP 17 (coded 14, 15 and 17) were provided by the National Autonomous Institute of Agricultural Research from Ecuador (INIAP, Ecuador), whilst commercial BR varieties SLF09 and F50 (coded 09 and 50) were supplied by a food processor (Procesadora Nacional de Alimentos C.A., INDIA-PRONACA). All varieties were grown in the coast area of Ecuador (Guayaquil) and showed particular features such as long grain and translucent center.

Sample Preparation

Germination processes were carried out as described previously [17]. Briefly, 50 g of BR grains were firstly washed in 0.1% (v/v) sodium hypochlorite and then imbibed in deionized water (1:5, w/v) at 28 °C for 24 h. Subsequently, grains were drained and placed in a germination cabin model EC00-065 (Snijders Scientific, Netherlands) in a relative humidity >90 % for 48 h and 96 h at 28 °C and 34 °C and darkness. Samples were freeze-dried, grounded, and stored in sealed plastic bags under vacuum conditions at 4 ºC until further analysis. Every germination batch was performed in triplicate.

Determination of Proteins, Fat, Ash, Dietary Fiber and Available Carbohydrates

Nitrogen was determined according to AOAC 984.13 [18] and protein content was calculated using 5.95 as conversion factor. Fat (AOAC 922.06), ash (AOAC 923.03) and soluble, insoluble and total DF (AOAC 985.29, AOAC 991.42 and AOAC 991.43, respectively) were also determined [19]. Available carbohydrates were estimated by difference: 100 – (% proteins + % fat + % water + % dietary fibre + % ash) [20].

Determination of Phytic Acid

The accurate photometrical Haug and Lantzsch´s determination of phytic acid phosphorous was used [21]. Absorbance was read at 540 nm in a microplate reader (BioTek Instruments, Winooski, VT, USA) (BioTek Instruments).

Determination of Degree of Proteolysis and Peptide Content

Degree of proteolysis was determined by the analysis of total and released free amino groups (FAG) in germinated samples. FAG were measured in BR and GBR by addition of 2,4,6-trinitrobenzenesulphonic acid (TNBS) as previously described [22]. Total FAG were also determined in BR and GBR samples previously hydrolyzed using 6N HCl for 24 h at 130 ºC. Degree of proteolysis was calculated as follows: DH (%) = [(FAG in germinated grain-FAG in raw grain)/ Total FAG] x100

Statistical analysis

Values are expressed as mean ± SD from independent germination experiments analyzed in duplicate. ANOVA and Duncan´s multiple range comparisons were used to assess differences at confidence level of 95% ($P\leq0.05$). Level of significance for temperature and time effects were calculated by statistical-t for each component ($P\leq0.05$) and positive or negative correlations were identified by the regression coefficient sign. Statistical analyses were performed by Statgraphics Centurion XVI software, version 16.1.17 (Statistical Graphics Corporation, Rockville, MD).

Results and discussion

Effect of germination conditions on the proximate composition of BR varieties

Figure 1 shows the proximate composition of crude, soaked (S) and germinated (GBR) brown rice. In crude grains, protein content ranged from 7.9 to 9.9 % dry matter (d.m.) (Figure 1A). These values were slightly higher than those previously reported in commercial BR [14,23]. Differences in BR protein content could be explained by intra-varietal genetic diversity, edaphoclimatic conditions and harvesting/storage management. BR soaking (24h at 28ºC) did not affect the protein content with exception of cv. GO (8% reduction, Figure 1A). Protein losses during rice soaking have been previously reported [13] and higher temperature led to lower protein content. Germination process affected differently the protein content depending on BR variety. In general, germination did not cause relevant changes in protein content; however, var. 50 germinated at 28 ºC for 4 days showed a significant increase (11%). In general, the largest protein content was observed in GBR var. 14, 15 and 09. BR cv. GO and var. 14 showed a negative correlation for germination time and a positive correlation for temperature ($P\leq0.05$) and var. 17 showed only a negative correlation for germination time ($P\leq0.05$). Decreased protein content is related to increased amino acid content as consequence of proteases activation [24]. This effect causes protein solubilization and its further leaching during radicle protrusion [13]. In addition, other studies showed an increased protein content in germinated seeds attributed to protein biosynthesis during germination, therefore, the protein content depends on the balance between protein degradation and protein biosynthesis during germination [23].

The content of available carbohydrates in crude BR ranged from 78.3-84.3 % d.m. (Figure 1B), results that agree with the literature [14,23]. BR soaking caused a 10% increase in the carbohydrates content ($P\leq0.05$) for cv.

GO, while it remained unchanged in var. 14, 17 and 50 and it underwent a slight decrease in var. 15 and 09 ($P \leq 0.05$). Germination brought about a decrease in the available carbohydrate content in all varieties studied. According to statistical analysis, carbohydrates content was negatively affected ($P \leq 0.05$) by both germination time and temperature and only var. 09 showed a negative correlation ($P \leq 0.05$) with germination time. In general, GBR cv. GO and INIAP varieties provided larger available carbohydrates than INDIA-PRONACA ones. Changes in the available carbohydrates can be attributed to the increased activity of endogenous α-amylase during germination [14,24] causing the hydrolysis of native starch and of the release of reducing sugars that are used as source of energy for the growing seedling [10]. The long-term consumption of GBR in type 2 diabetes patients as staple food was useful in improving blood glucose and lipid levels [25].

Commercial INDIA-PRONACA var. 09 and 50 presented higher lipid content (2.9 and 3.6 % d.m.) than INIAP var. 14, 15 and 17 (2.2-2.5 % d.m.), and cv. GO contained an intermediate lipid value (2.65 % d.m) (Figure 1C), values that are consistent with a previous study [26] that found palmitic, oleic and linoleic acids as the major fatty acids (80%). BR soaking led to a lipid increase in var. 15 and 09 ($P \leq 0.05$) and a noticeable lipid reduction ($P \leq 0.05$) in cv. GO and var. 17 and 50. Consequently, during germination lipid content underwent a different behavior depending on BR variety and germination conditions. Temperature was positively correlated with lipid content ($P \leq 0.05$) in all BR varieties, except for var. 15, while germination time was also positively correlated with lipid content for cv. GO and var. 15, 17 and 50. Decreased lipid content observed after germination has been ascribed to increased lipase activity to generate energy during seedling growth [27]. On the other hand, during germination the biosynthesis of lipids occurs, as it has been described for unsaturated and polyunsaturated fatty acids [23]. In addition, γ-oryzanol is the principal component of unsaponificable lipid fraction [28] that, along with tocopherols and tocotrienols, contributes to the nutritive quality and health promoting properties associated to GBR [4]. In general, INDIA-PRONACA GBR var. 09 provided larger lipid content than those from INIAP varieties and cv. GO.

Ash content of studied BR varieties ranged from 1.3 to 1.5% d.m. (Figure 1D), results that falls within those reported in the literature [14,23]. Ash content decreased ($P \leq 0.05$) in soaked BR, except of var. 09 where no significant differences were found. During germination, time and temperature affected ($P \leq 0.05$) ash content and negative correlations were found for cv. GO and INIAP varieties, whilst only germination time affected negatively ($P \leq 0.05$) to INDIA-PRONACA ones. Decreased ash content could be explained by lixiviation losses during soaking and watering, [23], nevertheless GBR still have a considerable amount of ash which reflects its mineral content, being INDIA-PRONACA varieties the ones with the largest ash content.

Effect of Germination on the Contents of Soluble and Insoluble Dietary Fiber

Table 1 shows the content of total dietary fiber (TDF) and their soluble (SDF) and insoluble (IDF) fractions in Ecuadorian crude, soaked (S) and germinated (GBR) brown rice. TDF did not exceed a 5 % d.m. in commercial INIAP var. 14, 15, 17 and cv. GO, whilst INDIA-PRONACA varieties provided around 8 % d.m, and SDF contributed with 72-75% and 66-68%, respectively. TDF content depends on BR variety and harvesting conditions [29]. Although the soaking process led to a slight ($P \leq 0.05$) increase of SDF and IDF in some studied BR varieties, the highest increase was observed after germination (Figure 2). SDF and IDF content of INIAP varieties were positively correlated ($P \leq 0.05$) with germination time and temperature and the highest TDF values were obtained for GBR var. 14 and 17 at 34°C for 96h (10 g/100g d.m.). INDIA-PRONACA varieties were positively affected by germination conditions and maximum TDF contents were reached in GBR var. 09 at 28 °C for 48h and GBR var. 50 at 28 ºC for 96h (13.6 and 13.6 % d.m., respectively). It is noteworthy that GBR var. 15 suffered the largest increase in SDF and IDF during germination at 34 ºC for 96 h (from 2.9 to 5.1 and 0.9 to 3.7% d.m., respectively), although the maximum values were found in var. 09 and 50 (Figure 2) in which SDF and IDF contributed similarly to TDF content (45-55%). It has been shown that TDF increases during BR germination [23]. In addition, germination impact on fiber fractions and SDF/IDF ratio depends on the variety and processing conditions [30,31]. It has been shown that the content of IDF can suppress post-pandrial glucose level after intake of soaked BR [16].

Effect of Germination on the Content of Phytic Acid

The content of phytic acid in crude, soaked (S) and germinated (GBR) varieties is collected in Table 2. Levels of phytic acid in crude BR were rather similar (from 1.15 % d.m in cv. GO to 1.5 % d.m. in var. 15). These results match with previous data found in BR var. Kenjian 90-31 [32] and are lower than those in BR cv. Ilpumbyeo [26]. Phytic acid content decreased ($P \leq 0.05$) after soaking in most Ecuadorian varieties (from 15 % in var. 50 to 48 % in var. 15), whilst no significant differences were observed in BR var. 09. Germination temperature and time negatively correlated with phytic acid content in all varieties, except for var. 09 in which phytic acid was only affected by germination time ($P \leq 0.05$). Interestingly, the lowest phytic acid content was obtained at 34°C for 96 h in all Ecuadorian BR varieties where losses between 32% for var. 09 and 80% for cv. GO were found. This effect has been attributed to increased endogenous phytase activity that releases phosphorous and lower myoinositol phosphates and, additionally to phytic acid lixiviation and further leaching into water [11]. From the nutritional point of view, phytic acid is associated with the mineral-related deficiency in humans, as well as protein and lipid availability. However, phytic acid could contribute to fight against a variety of cancers, diabetes, atherosclerosis and coronary heart diseases [33].

Effect of Germination on the Degree of Protein Hydrolysis

Figure 2A shows the degree of protein hydrolysis (DH) in soaked (S) and germinated (GBR) brown rice varieties. In general, proteolysis was negligible during soaking. Larger germination time and temperature led to higher proteolysis. The highest DH value (58.5%) was observed in var. 50 followed by var. 17 (48.9%) and cv. GO (48. 0%). Most of BR varieties showed a positive correlation between DH and germination time and temperature ($P \leq 0.05$). On the contrary, DH in cv. GO correlated negatively with germination temperature. During germination proteolysis occurred as consequence of increased protease activity that results in the release of peptides and free amino acids [24]. These effects are reported to improve protein digestibility of BR [14]. In order to confirm this fact, peptide content in crude BR and their respective processed grains was obtained (Figure 2B). Soaking led to a slight ($P \leq 0.05$) decrease in peptide content, except for var. 17 that showed a significant ($P \leq 0.05$) increase and var. 50, where no significant differences were found. Germination temperature and time affected positively ($P \leq 0.05$) peptide content, except for var. 09 in which germination temperature was negatively correlated with peptide content ($P \leq 0.05$). INDIA-PRONACA var. 09 showed the largest peptide content that can be ascribed to its large protein content (Figure 1A) and a noticeable DH (Figure 2A). In this variety the largest peptide content was obtained after 96 h of germination (3.24 and 4.7 g/100g d.m at 28 and 34 ºC, respectively). Bioactive peptides may be released as consequence of proteolysis that takes place during seed germination [34]. So far, enzymatic hydrolysis and fermentation are the biotechnological processes explored to produce antioxidant peptides from rice proteins [35], however, no information has been found on rice germination and related bioactive amino acid sequences. Thus, our group is currently performing further research to identify peptides from GBR with biological activities.

Conclusions

This study shows that germination of brown rice is a natural process of improving its nutritional quality; however, the extent of these positive effects depends on BR variety, germination time and temperature. Since germinated brown rice is a promising food choice to form part of healthy and sustainable diets of the population worldwide, cost-effective germination conditions such as 28 ºC and 48 h are recommended to enhance brown rice nutritional quality attributes.

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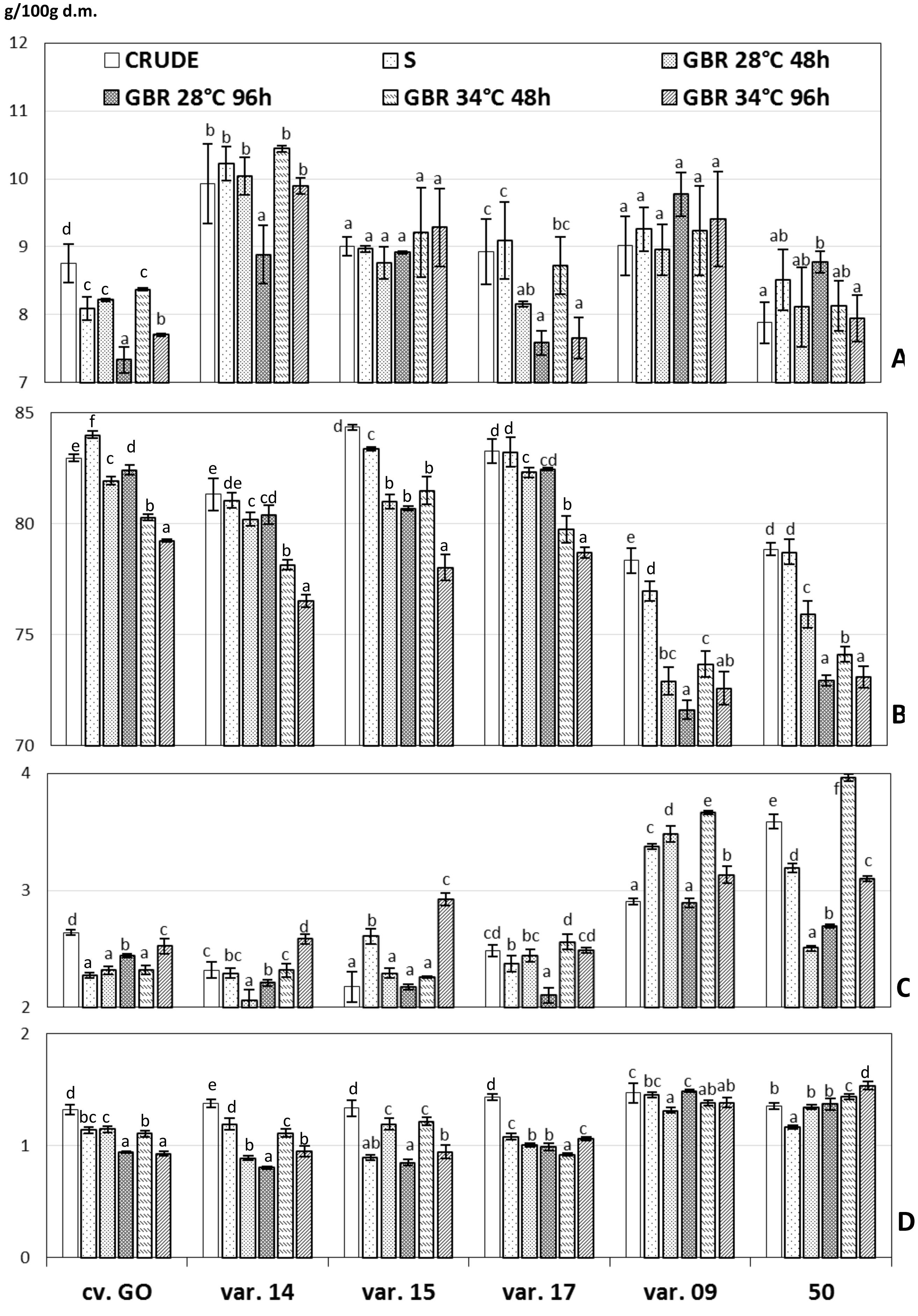

Figure 1. Content of protein (A), available carbohydrates (B), fat (C) and ash (D) of crude, soaked (S) and germinated (GBR) brown rice grains. Bars indicate mean values (g/100 g dry matter) and error lines indicate standard deviations. The same letter indicate no significant difference among mean values within a rice variety (P≤0.05 according to Duncan´s test).

Table 1. Phytic acid content (g/100g d.m) in crude, soaked (S) and germinated (GBR) Ecuadorian brown rice.

Treatment	Temperature (º C)	Time (h)	cv. GO	var. 14	var. 15	var. 17	var. 09	var. 50
Crude	---	---	1.15±0.02^{f}	1.35±0.06^{c}	1.52±0.04^{e}	1.26±0.0^{f}	1.19±0.04^{e}	1.25±0.05^{c}
S	28	24	0.84±0.02^{e}	0.83±0.03^{b}	0.80±0.06^{d}	0.95±0.03^{e}	1.16±0.03^{e}	1.06±0.03^{b}
GBR	28	48	0.71±0.02^{d}	0.80±0.05^{b}	0.68±0.03^{c}	0.68±0.08^{d}	0.93±0.03^{c}	1.00±0.04^{b}
	28	96	0.51±0.03^{b}	0.50±0.04^{a}	0.56±0.02^{b}	0.33±0.04^{b}	0.86±0.03^{b}	1.04±0.06^{b}
	34	48	0.62±0.02^{c}	0.50±0.04^{a}	0.72±0.05^{c}	0.53±0.06^{c}	1.04±0.04^{d}	1.02±0.03^{b}
	34	96	0.23±0.05^{a}	0.53±0.03^{a}	0.49±0.02^{a}	0.27±0.05^{a}	0.81±0.06^{a}	0.80±0.08^{a}

Data are the mean values ± standard deviation of three independent experiments (n=3). The same superscript indicates no significant difference among mean values within a column (P≤0.05 according to Duncan´s test).

Table 2. Dietary fiber content (g/100g d.m) in crude, soaked (S) and germinated (GBR) Ecuadorian brown rice.

Treatment	Temperature (ºC)	Time (h)	cv. GO	var. 14	var. 15	var. 17	var. 09	var. 50
Soluble dietary fiber								
Crude	---	---	3.10±0.11^{a}	3.63±0.08^{a}	2.90±0.19^{a}	3.19±0.05^{a}	5.16±0.09^{a}	4.85±0.16^{a}
S	28	24	3.11±0.18^{a}	3.84±0.13^{b}	3.03±0.07^{a}	3.16±0.13^{a}	5.33±0.06^{b}	4.81±0.13^{a}
GBR	28	48	3.52±0.08^{b}	4.04±0.08^{c}	3.08±0.03^{ab}	3.40±0.04^{b}	6.13±0.08^{c}	5.81±0.05^{b}
	28	96	3.71±0.07^{bc}	4.30±0.07^{d}	3.29±0.16^{b}	3.49±0.07^{b}	6.39±0.04^{d}	6.53±0.11^{d}
	34	48	3.77±0.11^{c}	4.30±0.08^{d}	3.58±0.19^{c}	3.89±0.07^{c}	6.23±0.03^{c}	6.20±0.06^{c}
	34	96	5.34±0.04^{d}	5.11±0.12^{e}	5.11±0.08^{d}	5.55±0.07^{d}	6.12±0.07^{c}	6.30±0.08^{c}
Insoluble dietary fiber								
Crude	---	---	1.21±0.06^{a}	1.42±0.08^{a}	0.96±0.10^{a}	1.15±0.12^{a}	3.80±0.11^{a}	3.74±0.10^{a}
S	28	24	1.37±0.06^{b}	1.40±0.05^{a}	1.17±0.06^{a}	1.36±0.02^{b}	4.13±0.04^{b}	3.86±0.04^{a}
GBR	28	48	2.85±0.15^{c}	2.75±0.12^{b}	3.09±0.19^{c}	2.78±0.13^{c}	7.47±0.17^{e}	6.78±0.12^{c}
	28	96	3.14±0.11^{d}	3.40±0.10^{c}	3.76±0.11^{d}	3.27±0.14^{d}	7.09±0.08^{d}	7.06±0.07^{d}
	34	48	4.13±0.03^{e}	3.66±0.22^{d}	2.65±0.11^{b}	4.27±0.08^{e}	5.85±0.05^{c}	5.85±0.11^{b}
	34	96	4.26±0.08^{e}	4.94±0.08^{e}	3.70±0.13^{d}	4.50±0.08^{f}	7.02±0.08^{d}	7.18±0.09^{d}

Data are the mean values ± standard deviation of three independent experiments (n=3). The same superscript indicates no significant difference among mean values within a column (P≤0.05 according to Duncan´s test).

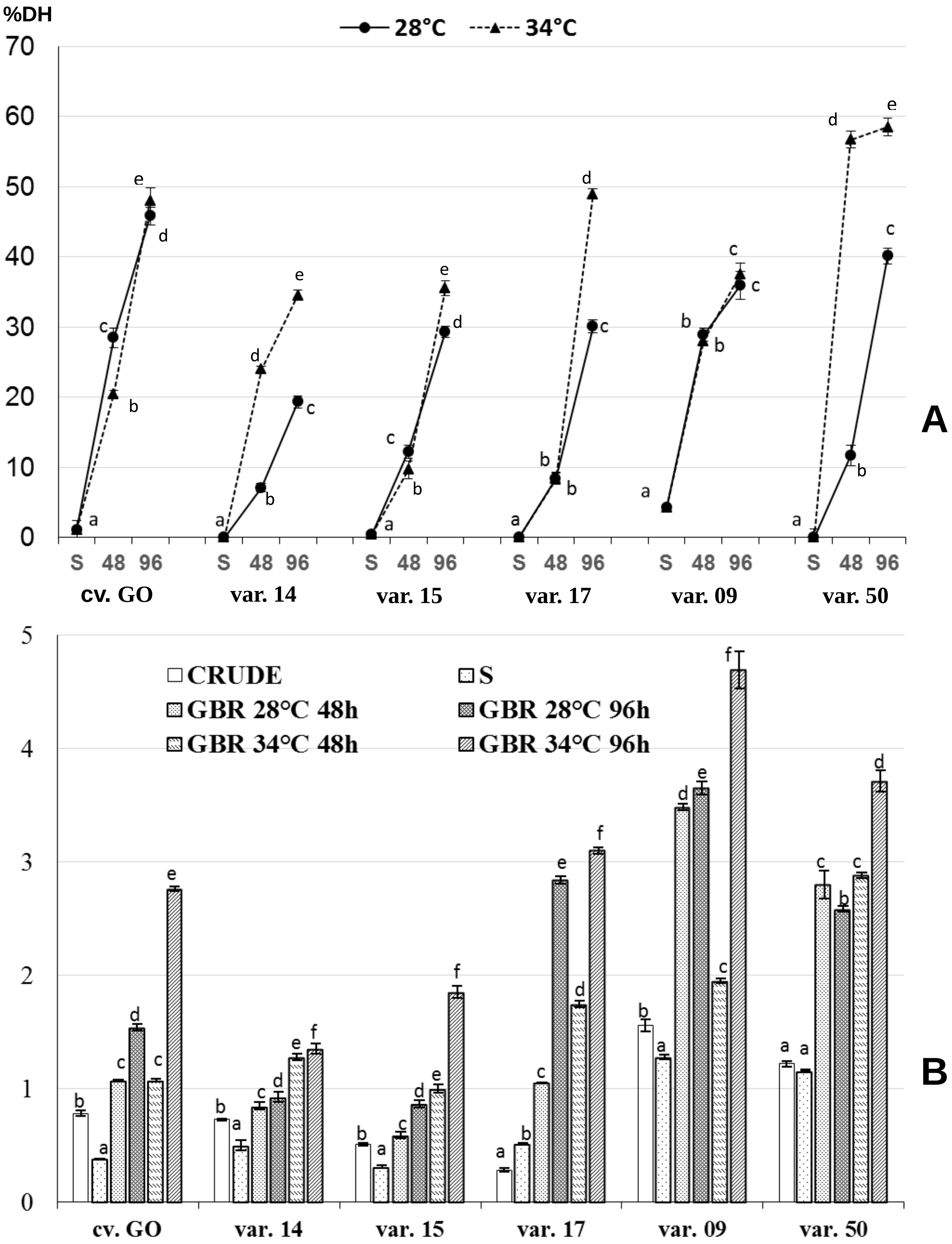

Figure 2. Panel A: Degree of hydrolysis (%DH) of soaked (S) and germinated Ecuadorian brown rice for 48 h (48) and 96 h (96). Panel B: Peptide content (g/100g d.m.) in crude, soaked (S) and germinated (GBR) Ecuadorian brown rice. The same letter indicates no significant difference within a variety (P≤0.05, according to Duncan´s test).