[Frontiers in Bioscience 16, 1609-1618, January 1, 2011]

Role of cereal type and processing in whole grain *in vivo* protection from oxidative stress

Andrea Gianotti[1], Francesca Danesi[1], Vito Verardo[1], Diana Isabella Serrazanetti[1], Veronica Valli[1], Alessandra Russo[1], Ylenia Riciputi[1], Nadia Tossani[2], Maria Fiorenza Caboni[1], Maria Elisabetta Guerzoni[1], Alessandra Bordoni[1]

[1]Department of Food Sciences - University of Bologna, Viale Fanin, 44, 40127 Bologna (BO) Italy, [2]Department of Biochemistry, G. Moruzzi, University of Bologna, Via Irnerio, 48 - 40126 Bologna (BO) Italy

TABLE OF CONTENTS

1. ABSTRACT

The reduced risk of chronic diseases related to whole grain consumption is in part attributed to their high antioxidant content. Many studies have been performed on the *in vitro* antioxidant capacity of cereals, but *in vivo* studies are necessary. We have evaluated and compared the effect of whole grain durum wheat bread and whole grain Kamut® khorasan bread on the oxidative status in rats. Two different bread-making processes were used for whole grain Kamut® khorasan, sourdough and baker's yeast. After 7 weeks on the experimental diets rats were divided into two subgroups, one receiving an oxidative stress by doxorubicin injection. Our results evidenced both wheat durum and Kamut® khorasan as good sources of antioxidants, and a lower oxidative state in rats fed the cereal-based diets. Furthermore, Kamut® khorasan bread fed animals had a better response to stress than wheat durum fed, especially when a sourdough bread was supplied. Although further studies are needed, data herein reported suggest whole grains, particularly whole ancient grains, as a safe and convenient way of increasing antioxidant protection.

2. INTRODUCTION

The increased consumption of whole grains and whole grain products has been associated with reduced risk of developing chronic diseases (1). Epidemiological studies also confirm that high whole grain intake protects against cancer, cardiovascular disease, diabetes, and obesity (2). Potential mechanisms for this protection are diverse, since whole grains are rich in nutrients and phytochemicals. First, whole grains are concentrated sources of dietary fiber, resistant starch and oligosaccharides, which escape digestion in the small intestine and are fermented in the gut, producing short-chain fatty acids (SCFA). SCFA lower colonic pH, serve as an energy source for the colonocytes and may alter blood lipids. These improvements in the gut environment may provide immune protection beyond the gut (3).

Second, whole grains are rich in antioxidants, including trace minerals and phenolic compounds, which have been linked to disease prevention. Results of research (4, 5) have shown that the total phytochemical content and antioxidant activity of whole grains have been previously

underestimated, and that whole grains contain more phytochemicals, mostly in bound form, than previously reported. In addition, whole grains contain unique phytochemicals that complement those in fruits and vegetables (6). These compounds include phytate, phyto-estrogens such as lignans, plant stanols and sterols, vitamins, and minerals.

The diversity of the active constituents in the different whole grain products and the complexity of the metabolic responses to each of them make it difficult to understand the mechanism by which whole grain cereals contribute to the protection against oxidative conditions and related diseases (7). Actually, the *in vitro* antioxidant capacity of cereals and their constituents is only an approximate reflection of their *in vivo* antioxidant effect. Differences in antioxidant solubility and/or bioavailability within the digestive tract and the metabolism and/or conjugation of antioxidant compounds must be considered (7), and *in vivo* studies are necessary before drawing conclusions.

This lack of information is particularly evident for the so-called "ancient wheat", which is increasingly interesting to both the food industry and consumers as a raw material or ingredient that feature natural and enhanced nutritional properties. The ancient wheat representing the main alternative cereals are Kamut® khorasan wheat, spelt (*Triticum spelta)*, einkorn (*Triticum monococcum)* and emmer (*Triticum dicoccum)*. Kamut® is a registered trademark used in marketing products of the organically grown QK-77 variety of *Triticum turgidum*, *ssp. turanicum*, whose common name is khorasan (8). Recently Khlestkina *et al.* (9) suggested that khorasan could be the outcome of a natural hybridization event between *T. durum* and *T. polonicum*, which took place in the Fertile Crescent. A few studies regarding the nutritional value of Kamut® khorasan are present in the literature, but its high carotenoid content (10) and the low allergenic potential of ancient wheats (11) make it potentially interesting.

The aim of the present study was to evaluate *in vivo* the effectiveness of a whole grain food product as a source of antioxidant, evidencing possible differences between modern and ancient wheat. At present, most studies regarding the antioxidant capacity of cereals are *in vitro* studies, based on unprocessed or partially processed cereals (whole meal or refined flour, germ, bran) rather than on cereal food products. Among these products, we chose bread since it is the most common, worldwide consumed one. We evaluated the effect of bread made from whole grain modern durum wheat and bread made from whole grain Kamut® khorasan on the oxidative status in rats. Durum wheat was used instead of soft wheat because of its close taxonomic relationship to khorasan. Furthermore, since sourdough fermentation is widely used in the biotechnology of baked goods because of the many advantages it offers with respect to the baker's yeast, two different bread-making processes were used for whole grain Kamut® khorasan, i.e. one using sourdough and one using only baker's yeast. The advantages of sourdough process are mainly related to the metabolic activities of lactic acid bacteria: lactic and acetic acid formation, proteolysis, synthesis of flavor compounds (12-14), prevention of microbial contamination, decrease of starch staling. In the case of whole grains, sourdough may influence the levels and bioavailability of the bioactive compounds (15-17).

In the light of the aim of this study, planning the study design we discarded the possibility of adding whole grain phytochemicals to a standard diet during its preparation for different reasons. First, whole grains contain many different phytochemicals, and the synergism of antioxidant components is well documented. The supplementation of the diet with one or more bioactives cannot give an overall vision of the possible impact of a certain food on antioxidant defenses. Furthermore, the addition of one or more phytochemicals to the standard diets implies an *a priori* choice of the most active compounds. To date, there are not enough data for attributing the health benefit of whole grains to specific bioactives. The use of whole grains for the preparation of a modified pellet diet was also discarded because it would have anyway modified the overall composition of the experimental diets, and the addition of a vitamin and mineral mix would have probably masked the possible effect of whole grain antioxidant compounds. Therefore we decided to feed animals exclusively with the different experimental breads. This approach allowed to: (i) exacerbate the possible effect of whole grain breads, avoiding any interference derived from bioactives derived from other sources; (ii) evidence the effect of a food (bread) usually present in the human diet, considering also changes in its nutritional properties that can derive from processing of raw material; (iii) examine differences related to different bread-making processes.

Although we were aware that the study design was uncommon, and the experimental breads could not completely match the nutritional requirements of rats, in our opinion this approach was the only one allowing to reach the objective of the study, i.e. the demonstration of the effectiveness of whole grain bread as source of antioxidants.

After 7 weeks on the experimental diets, animals were submitted to an oxidative stress by intraperitoneal injection of doxorubicin (DOX), and the response to stress was evaluated by measuring plasma total antioxidant activity (TAA) and concentration of reactive oxygen molecules (ROMs). The effect of the experimental diets on growth and blood glucose level were also determined. Age-matched rats were also housed and fed a standard diet for the whole duration of the experimental protocol.

3. MATERIALS AND METHODS

3.1. Chemicals and reagents

Doxorubicin was a kind gift of Ebewe (Rome, Italy). All chemicals and solvents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and were of the highest analytical grade.

3.2. Diet preparation

Wheat (*Triticum durum*) bread (WB), Kamut® khorasan bread (KB), and Kamut® khorasan sourdough bread (SKB) were obtained by mixing whole grain organic durum wheat flour or whole grain organic Kamut® khorasan wheat flour with distilled water (45.4%, 50.0% and 49.2% w/w on flour basis, respectively). Sunflower oil (12.72% w/w on flour basis) and baker's yeast (*Saccharomyces cerevisiae*) (2% w/w on flour basis) were added to all the doughs used in this experiment. In SKB a mother dough (17.2% w/w on flour basis) was also added. The mother sourdough was obtained by daily propagation and the manufacture included two steps of long and short fermentation for ~8 h and 4 h respectively at 22°C and 30°C. The doughs were leavened for 1.5 hours at 30°C and finally baked for 25 min at 215°C. The baked breads were sliced and cut into little cubes (volume 1 cm^3), put in vacuum sealed plastic bags and frozen until their use. One bag for each diet was thawed daily to feed the rats.

The standard diet contained proteins (21% w/w), lipids (8% w/w), and carbohydrates (61.5% w/w) in the normal nutritional range for rats, as well as appropriate amounts of vitamins and minerals.

3.3. Bread composition

Total carotenoids were determined in 80% acetone extracts by the method of Lichtenthaler *et al.* (18). Total folic acid content was determined according to the AOAC official method 944.12 according to Koontz *et al.* (19), and vitamin E content was analyzed by HPLC according to Gueguen *et al.* (20). Soluble, insoluble and total dietary fiber content was estimated according to the method described by Asp *et al.* (21).

Selenium (Se) analyses were carried out by Inductively Coupled Plasma Mass Spectrometry (ICPMS) according to Bryszewska *et al.* (22) on samples digested with nitric acid and finished with hydrochloric acid. Finally, moisture, ash, protein, and fat were evaluated according to the standard AACC methods (23).

Phenolics were extracted from samples at room temperature using a mixture of ethanol/water according to Verardo *et al.* (24). Total phenolic compounds (TPC) were determined at 750 nm using the Folin-Ciocalteu spectrophotometric method according to Verardo *et al.* (24). To assess TPC a gallic acid calibration curve was made. RP-HPLC analysis was performed according to Verardo *et al.* (24) by a HPLC-DAD-MS (1100 Series, Agilent Technologies, Palo Alto, California, USA). Calibration curves for both ferulic acid and quercetin were constructed from 1-500 microg/mL, respectively, at 6 concentration levels for each compound, plotting peak area *vs.* analyte concentration. The HPLC analysis was replicated three times for each extracts and calibration points (n=3).

3.4. Animals

Sixty-four male Wistar rats, aged 30 days, were used. Animals were housed in individual cages in strictly controlled conditions of temperature (20 +/- 2°C) and humidity (60-70%), with a 12 hour dark-light cycle.

Animals were randomly divided into four groups, each receiving one of the following diets: 1. standard chow diet (SD); 2. wheat bread (WB); 3. Kamut® khorasan bread (KB); 4. Kamut® khorasan sourdough bread (SKB). In the three experimental groups, rats were fed exclusively the different types of bread. Water and food were provided *ad libitum*. Food consumption was measured every day, and rat body weight (b.w.) every week.

The dietary treatment lasted for 7 weeks, then rats of each group were randomly divided into two subgroups, the first one receiving intraperitoneally 10 mg/kg b.w. of doxorubicin (DOX) in a single dose, the second one similar volumes of NaCl 0.9% (w/v) in distilled, apyrogen water solution. DOX is an anthracycline antibiotic, widely used as anticancer agent. Despite its high antitumor activity, its use in clinical chemotherapy is limited because of diverse toxicities. Oxidative damage to membrane lipids and other cellular components is believed to be a major factor in the DOX toxicity, which is mediated by the formation of an iron-anthracycline complex that generates free radicals (25). DOX was administered intraperitoneally to escape the interference due to the gastro-intestinal tract (26-29).

After DOX injection, rats kept eating the same diet for 36 hours, then, after a 12 hour starvation, they were sacrificed. Blood was sampled by intracardiac withdrawal, plasma immediately separated by centrifugation and stored in separated aliquots at -20°C until analysis. The Animal Care Committee of the University of Bologna approved the study (Prot. 50932-X/10).

3.5. Plasma total antioxidant activity (TAA)

TAA was measured in plasma using the method of Re *et al.* (30), on the basis of the ability of the antioxidant molecules in the sample to reduce the radical cation of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), determined by the decolorization of $ABTS^{\bullet+}$, and measured as the quenching of the absorbance at 734 nm. Values obtained for each sample were compared to the concentration-response curve of the standard trolox solution and expressed as micromoles of trolox equivalent (TE)/mL.

3.6. Plasma peroxide level

The concentration of reactive oxygen metabolites (ROMs) in plasma was measured by applying the d-ROMs test (Diacron, Grosseto, Italy) as reported by Danesi *et al.* (26). This test is based on the ability of transition metals to react with peroxides by the Fenton reaction. The reaction produces free radicals that, trapped by an alchilamine, form a colored compound detectable at 505 nm. Values obtained for each samples were compared to standard (H_2O_2), and expressed as microg H_2O_2/mL.

3.7. Plasma glucose estimation

Plasma glucose level was determined by the glucose oxidase enzymatic method (31). Briefly, glucose present in the sample is oxidized by the enzyme glucose oxidase to gluconic acid with the liberation of hydrogen peroxide (H_2O_2), which reacts by peroxidase with 4-aminophenazone and phenol giving a colored compound

Table 1. Nutritional composition of experimental breads

Component	WB	KB	SKB
Energy (Kcal/100g)	347 +/- 17	338 +/- 12	323 +/- 15
Water (g/100g)	19.0 +/- 0.7 [a]	20.1 +/- 0.6 [b]	22.1 +/- 0.6 [a,b]
Protein (g/100g)	9.6 +/- 0.5 [a,b]	11.2 +/- 0.5 [a]	11.4 +/- 0.6 [b]
Fat (g/100g)	10.3 +/- 0.9	11.0 +/- 1.0	9.8 +/- 0.9
Soluble carbohydrates (g/100g)	54.0 +/- 2.4 [a]	48.5 +/- 2.2	47.30+/- 2.0 [a]
Soluble fiber (g/100g)	0.5 +/- 0.3	0.7 +/- 0.2	0.5 +/- 0.2
Insoluble fiber (g/100g)	5.7 +/- 1.0	7.1 +/- 0.9	7.6 +/- 1.0
Ash (g/100g)	0.89 +/- 0.13 [a,b]	1.41 +/- 0.11 [a]	1.41 +/- 0.12 [b]
Vitamin E (microg/100g)	1500 +/- 80 [a,b]	1060 +/- 95 [a,c]	2184 +/- 90 [b,c]
Beta-carotene (microg/100g)	23.1 +/- 2.1 [a]	16.0 +/- 2.1 [a,b]	22.0 +/- 3.0 [b]
Folic acid (microg/100g)	40.5 +/- 2.5 [a,b]	29.5 +/- 2.1 [a,c]	21.3 +/- 1.9 [b,c]
Total polyphenols (mg gallic acid/100g)	37.6 +/- 0.6 [a,b]	44.9 +/- 1.7 [a]	43.8 +/- 0.5 [b]
Selenium (microg/100g)	0.089 +/- 0.040 [a,b]	0.892 +/- 0.050 [a]	0.923 +/- 0.040 [b]

Breads were analyzed as reported in Methods. Data are means +/- s.d. Statistical analysis was by one way ANOVA using Tukeys' as post test. Similar letters indicate statistical significance (at least $p<0.05$).

Table 2. Phenol content and composition of the two experimental breads

Compound	$[M-H]^-$	Reference	WB	KB	SKB
Ferulic acid derivative	323	32	n.d.	2.30 +/- 0.01 [c]	2.90 +/- 0.10 [c]
Isoshaftoside	563	33	8.30 +/- 0.20 [a]	10.40 +/- 0.60 [a,c]	7.80 +/- 0.40 [c]
p-coumaric acid	163	33	2.10 +/- 0.10 [a,b]	1.90 +/- 0.01 [a,c]	2.40 +/- 0.01 [b,c]
Shaftoside	563	33	21.60 +/- 1.50 [a]	18.80 +/- 0.10 [a]	20.50 +/- 1.00
Dihydroferulic acid	385	33	n.d.	0.60 +/- 0.10	0.70 +/- 0.10
Ferulic acid	193	33	6.70 +/- 0.04 [a,b]	6.00 +/- 0.30 [a,c]	4.50 +/- 0.20 [b,c]
Apigenin-6-C-B-galactosyl-8-C-B-glucosyl-O-glucoronopyranoside	769	33	4.00 +/- 0.60 [a,b]	6.80 +/- 0.60 [a]	7.20 +/- 0.70 [b]
Total			42.60 +/- 2.34	46.71 +/- 1.50	46.0 +/- 1.60

Breads were analyzed as reported in Methods. Data are means +/- s.d. Phenolic acid content is expressed as mg ferulic acid/g d.w., flavonoid content as mg quercetin/g d.w. Statistical analysis was by one way ANOVA using Tukeys' as post test, except for ferulic acid derivative and dihydroferulic acid analyzed by the Student's t test. Similar letters indicate statistical significance (at least $p<0.05$).

which can be measured at 515 nm. Values obtained for each samples were compared to a standard curve obtained using glucose serial dilutions, and were expressed as mg/dL.

3.8. Statistical analysis

All data are reported as mean +/- standard deviation (s.d.). Statistical analysis was by the one-way ANOVA, using Tukey's as post test, or by the Student's t test.

4. RESULTS

The three experimental breads provided similar energy, fat, and fiber, while protein content was higher and soluble carbohydrates lower in breads prepared using Kamut® khorasan (Table 1). The concentration of all potentially antioxidant compounds appeared different in the three experimental breads. Total polyphenols and particularly selenium were significantly higher in KB and SKB than in WB, while folic acid presented higher values in WB (Table 1). Regarding vitamin E and beta-carotene, the lower content observed in KB than WB was increased by the sourdough baking.

Although the total amount of phenolic acids and flavonoids was similar in the three experimental breads their profile was significantly different (Table 2). The detected phenolic compounds have been previously described in wheat, therefore the identification of the different compounds was made comparing their UV and mass spectra with those reported in other works, as indicated in Table 2. Notably, dihydroferulic and other derivatives of ferulic acid were present in KB but not in WB.

Experimental rats grew significantly less, and their b.w. at the end of dietary treatment was about 50% than SD rats (Figure 1). This difference was in agreement with the lower food consumption observed in experimental animals (about 22 g/day in SD fed and about 12 g/day in experimental diet fed rats) (*data for food intake not shown*).

All experimental animals appeared frankly hypoglycemic, and DOX administration elevated their glucose plasma level (Figure 2). On the contrary, the oxidative stress did not affect glycemia in SD rats.

In basal conditions, no significant differences in plasma TAA were detected either among the different dietary groups or compared to SD animals (Figure 3). The oxidative stress caused by the intraperitoneal administration of DOX negatively affected plasma TAA in SD and WB fed rats ($p<0.01$ compared to the corresponding basal value), while it was ineffective in KB and SKB animals.

In basal conditions, ROM plasma level was significantly lower in rats fed with the experimental breads than in SD fed, SKB animals showing the lowest ROM concentration (Figure 4). In SD and WB rats DOX administration caused a significant increase in ROM plasma level, while in KB and SKB groups ROM concentration did not change with respect to corresponding basal conditions.

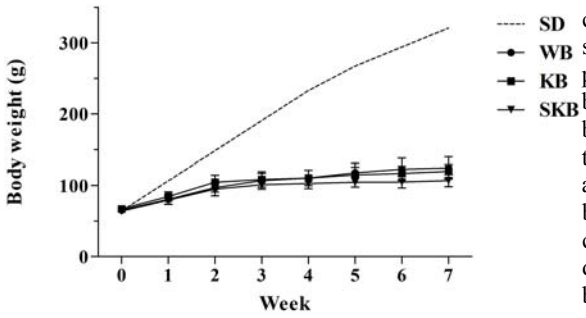

Figure 1. Body weight increase in rats fed the experimental diets. Each rat was weighted every week, at the same time in the morning. Data are means +/- s.d. of 12 rats in each group. The mean b.w. increase in pair matched rats fed the standard diet is also reported. Statistical analysis was by one-way ANOVA ($p<0.001$ at all time point except T0) using Tukey's as post test (SD *vs.* all experimental diets $p<0.001$).

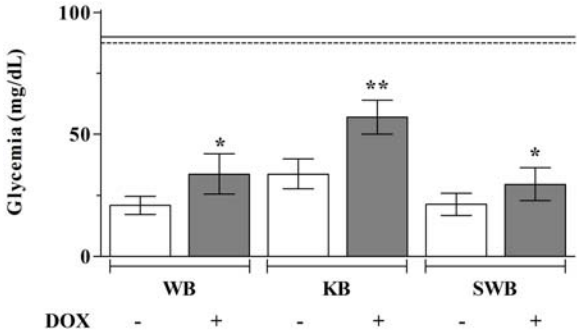

Figure 2. Blood glucose level of rats fed the experimental diets in basal and stressed condition. Blood glucose was determined as reported in Methods, and expressed as mg/dL. Data are means +/- s.d. of 6 rats in each group. Blood glucose level in basal (90.01 +/- 10.05 mg/dL, continuous line) and oxidative condition (87.45 +/- 11.32 mg/dL, dashed line) in pair matched rats fed the standard diet is also reported. Glycemia was significantly lower in experimental rats than in standard diet fed ones ($p<0.001$ by the one way ANOVA). Comparison among experimental rats in basal condition (white bars) was by the one-way ANOVA ($p<0.01$) using Tukey's as post test (WB *vs.* KB $p<0.01$; KB *vs.* SKB $p<0.01$). No difference in blood glucose level was detected in standard diet fed rats after DOX administration. In each experimental group, glycemia in stressed animals (grey bars) was compared with the corresponding basal condition (* $p<0.05$; ** $p<0.01$). Comparison among DOX treated experimental animals was by one-way ANOVA ($p<0.01$) using Tukey's as post test (WB *vs.* KB $p<0.01$; KB *vs.* SKB $p<0.01$).

5. DISCUSSION

Whole grains contain significant concentrations of antioxidant compounds that could contribute to dietary antioxidant intake, and many studies have evidenced that cereal products, particularly whole grain ones, have significant antioxidant potential *in vitro* (34). Studies on cereal products conducted on animals and humans remain scarce in view of the numerous *in vitro* studies, and this is paradoxical since most cereal antioxidants are only partly bioavailable (7). Moreover, most of the *in vitro* studies are based on unprocessed or partially processed cereals rather than on cereal food products, and no information is available on antioxidant capacity of Kamut® khorasan bread. In this investigation we have evaluated and compared in rats the effectiveness of whole grain modern durum bread and whole grain ancient Kamut® khorasan bread as antioxidant source. To exacerbate the effects of whole wheat and Kamut® khorasan, and to avoid bias, experimental diets consisted of bread only. An aged-matched rat group was fed a standard diet for the whole duration of the experiment. We were totally aware that the bread-based diets and the standard diet were not comparable. In this study, standard diet fed animals cannot be considered as control animals. They were just used to set the stage and to verify what happens, regarding the tested parameters, in pair-matched rats fed a normal diet.

The experimental diets were not completely balanced, although sunflower oil had been added during bread making to meet lipid and essential fatty acid requirements, and experimental rats of both groups grew significantly less than SD rats. This difference was related to the lower food consumption observed in experimental animals, and not to a different feed efficiency of the experimental diets. Our results are in agreement with the reported role that whole grains play in b.w. regulation, based on the effects that the components of whole grains have on hormonal factors, satiety, and satiation. In both clinical trials and observational studies the intake of whole grain foods was inversely associated with plasma biomarkers of obesity, including leptin concentrations. The fiber content of whole grains may also affect the secretion of gut hormones that may act as satiety factors (35).

Although smaller in size, all experimental rats appeared in a fair state of health, as assessed by a veterinary surgeon, having normal reactivity and behavior and no symptoms of malnutrition. The lower blood glucose level observed in bread-fed rats can be easily attributable mainly to the reduction in food consumption (36). Interestingly, a hypoglycemic effect has been observed in both normal and STZ-induced diabetic rats after consumption of an aqueous extract of *Triticum repens*, a plant found in the wild belonging to the Gramin family (37).

Notwithstanding the lower food intake, experimental diets were able to effectively match the requirement of exogenous antioxidants, as evidenced by plasma TAA which was similar in all groups in basal condition. This confirms *in vivo* that whole grain bread is a very good source of bioavailable antioxidants. It is particularly relevant considering that experimental rats were fed a not completely balanced diet, probably leading to insufficient intake of some nutrients. Relative deficiencies of many micronutrients are related to an increased oxidative status (38-41). In this light, whole grain

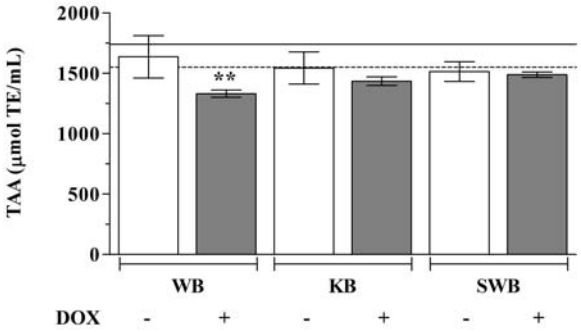

Figure 3. Plasma total antioxidant activity (TAA) of rats fed the experimental diets in basal and stressed condition. Plasma TAA was determined as reported in Methods, and expressed as micromol Trolox Equivalent (TE)/mL. Data are means +/- s.d. of 6 rats in each group. TAA in basal (1740 +/- 59 micromol TE/mL, dotted line) and oxidative condition (1550 +/- 35 micromol TE/mL, dashed line) in pair matched rats fed the standard diet is also reported. In basal condition, TAA was similar in standard diet and experimental diet fed rats (n.s. by the one way ANOVA), and among experimental rats (white bars) (n.s. by the one-way ANOVA). In oxidative condition, a significant decrease in TAA was detected in standard diet fed rats ($p<0.001$ by the Student's t test). In each experimental group, TAA in stressed animals (grey bars) was compared with the corresponding basal condition (** $p<0.01$ by the Student's t test). Comparison among DOX treated experimental animals was by one-way ANOVA ($p<0.001$) using Tukey's as post test (WB *vs.* KB and SKB $p<0.001$; KB *vs.* SKB $p<0.05$).

breads appeared particularly effective since they maintained antioxidant defenses at a normal level.

The oxidative stress caused by the intraperitoneal administration of DOX negatively affected plasma TAA in SD and WB fed rats ($p<0.01$ compared to the corresponding basal value), while it was ineffective in KB and SKB ones. As we observed in previous works in both cultured cardiomyocytes (42, 43) and rats (26, 27), TAA reduction after DOX administration could reflected a consumption of antioxidant molecules because of the adriamycin-induced increase in ROM production. Accordingly, ROM plasma level in oxidative condition increased in SD and WB animals (Figure 4). On the contrary, DOX administration did not cause either a decrease in plasma TAA or an increase in ROM concentration in KB fed animals.

The better response to oxidative stress observed in KB and SKB than in WB rats is hardly attributable to a single specific component, since the content of all the main antioxidants was different in the three experimental breads. WB, KB, and SKB also evidenced a different phenolic profile. Several studies demonstrated that ferulic acid and its derivatives are the major hydroxycinnamic acids detected in human and rat plasma after cereal consumption (44, 45), and many reports indicate that these compounds possess the greatest antioxidant capacity *in vitro* and the highest protective effect against oxidative stress *in vivo* (46-48). Ferulic acid derivatives and dihydroferulic acid were not identified in WB, and their content was higher in SKB than in KB.

Furthermore, KB and SKB selenium content was higher than in WB. Although all whole grain foods are considered valuable sources of selenium (3), in this study Se content was 10 fold higher in Kamut® khorasan based bread. Similar results have been recently reported by Piergiovanni *et al.* (49) who suggested that a high aptitude to uptake Se from soil could be associated to Kamut® khorasan. Selenium functions in the active site of a large number of selenium dependent enzymes which participate in the oxidative stress protection of cells (50). The high availability of selenium represents the single most important factor for biosynthesis of glutathione peroxidase (GPx) and other selenoproteins, and the very high intake of the micronutrient could have sustained the synthesis of these antioxidant enzymes in KB and SKB fed rats, therefore maintaining a high antioxidant activity even in stressed condition. This is in agreement with our previous works on GPx activity in liver and heart of Se supplemented rats (26, 27).

In the living organism, all antioxidants contribute in a synergistic way to the overall defense against oxidative damage, so it is conceivable that all experimental diets had an appropriate antioxidant profile able to maintain total antioxidant activity in basal condition, and the best profile of KB and SKB was evidenced only after DOX administration.

Many studies indicate that the protection against DOX-induced toxicity obtained by supplementation with different bioactives/foods can be attributed, at least in part, to antioxidant activity (28, 51). TAA can be considered as the summation of the interactions among various endogenous and exogenous antioxidants. Although a highest selenium intake has been related to the increase of plasma TAA (52-54), it is difficult to discriminate the contribution of an enhanced activity of plasma glutathione peroxidase and other selenoproteins to TAA level. Anyway, a high TAA level is considered important in the counteraction of the oxidative stress.

Oxidative stress is a term used to denote the imbalance between the concentrations of reactive oxygen and nitrogen species and the defense mechanisms of the body. In this light, the assays used for evaluation of "oxidative stress" can be divided in: (i) assays based on measuring the concentrations of oxidation products; (ii) assays based on measuring the concentration of antioxidants. A plethora of different assays are available for measurements of both oxidation products and antioxidants. Unfortunately, as reported by Dotan *et al.* (55), none of these methods can be defined as the most appropriate criterion for defining "oxidative stress" in universal terms. In this study, we evaluated the oxidative stress by measuring plasma TAA and ROMs. Plasma TAA is a widely used parameter considered as the summation of the interactions among various antioxidants, and ROM plasma level is reported as a valid indicator for

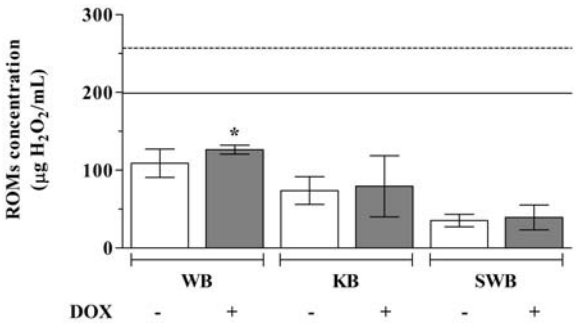

Figure 4. Plasma level of reactive oxygen molecules (ROMs) in rats fed the experimental diets in basal and stressed condition. Plasma ROMs level was determined as reported in Methods, and expressed as microg H_2O_2/mL. Data are means +/- s.d. of 6 rats in each group. ROMs level in basal (198.80 +/- 20.60 microg H_2O_2/mL, continuous line) and oxidative condition (257.10 +/- 8.70 microg H_2O_2/mL, dashed line) in pair matched rats fed the standard diet is also reported. ROMs level was significantly lower in experimental rats than in standard diet fed ones ($p<0.001$ by the one way ANOVA). Comparison among experimental rats in basal condition (white bars) was by the one-way ANOVA ($p<0.001$) using Tukey's as post test (WB *vs.* KB $p<0.01$; WB *vs.* SKB $p<0.001$; KB *vs.* SKB $p<0.01$). In oxidative condition, a significant increase in ROMs level was detected in standard diet fed rats ($p<0.01$ by the Student's t test). In each experimental group, ROMs level in stressed animals (grey bars) was compared with the corresponding basal condition (* $p<0.05$ by the Student's t test). Comparison among DOX treated experimental animals was by one-way ANOVA ($p<0.001$) using Tukey's as post test (WB *vs.* KB $p<0.05$; WB *vs.* SKB $p<0.001$).

oxidative damage measurements by different authors (56-58). Although these two assays, as well other assays, cannot universally predict the "oxidative status", they allow to evidence the effects related to the consumption of different whole grain breads. It is important to note that in our study results related to these two indicators were in total agreement, and a significant inverse correlation was found between TAA and ROM level (Pearson's correlation: -0.33, $p<0.05$). Actually, the role of experimental diets as good sources of antioxidants in basal condition was evidenced not only by TAA, but also by ROM plasma level, which was significantly lower in rats fed with the experimental breads than in SD ones. Besides antioxidant intake, these results could be also accounted to the lower food intake in experimental animals, since it is documented that energy restriction is a powerful intervention that slows the aging process, probably acting also through an attenuation of oxidative damage (59).

Although the type of wheat used for bread making (durum *vs.* Kamut® khorasan) appeared to be the main determinant of the observed protective effect, the sourdough fermentation process could also play a key role. In fact, although all animals fed Kamut® khorasan bread had a low ROM plasma level, in basal condition it was lower in SKB than in KB ($p<0.01$). The antioxidant activity of lactic-fermented foods has been widely described with *in vitro* assays (60), and it has been suggested that sourdough fermentation increases the levels of easy-extractable phenolic compounds (16). Furthermore, vitamin E content was twice as much in SKB than in KB. It is conceivable that this is related to both the presence of particular microorganisms and their role in fermentation process. Actually, in the end of fermentation process yeasts (mainly represented by *Saccharomyces cerevisiae*) and lactic acid bacteria (mainly belonging to *L. plantarum* species) accounted around to 7.8 and 7.9 log CFU/g respectively. Moreover SKB dough was characterized by a lower pH (3.96) in comparison to WB and KB (6.29 and 6.91 respectively), due to the sourdough fermentation. It has been reported that *S. cerevisiae* can significantly increase the total concentration of tocopherols and tocotrienols, depending on type of grain and process (61). This is particularly evident when grains are subjected to an enzymatic pre-treatment (amylases, proteases, glucanases). Therefore, the sourdough fermentation "pre-treatment" could have enhanced the vitamin E content in SKB. Regarding lactic acid bacteria, although they are not reported as vitamin E producers, their antioxidant ability in rat deficient in vitamin E has been described by Lin *et al.* (62). Beta-carotene content was also higher in SKB than in KB. Several Authors demonstrated the positive influence of fermentation (by yeasts and bacteria) on antioxidant compounds including vitamins C, E and carotenoids in protein based foods (63) and traditional fermented foods (64). It is conceivable that sourdough fermentation, causing the observed modification in SKB composition, has significantly contributed to the protective activity.

In conclusion, whole grain bread appears to be a good source of exogenous antioxidants and is able to effectively match the maintenance of normal plasma TAA in basal condition. The onset of an oxidative condition evidences differences related to raw material (whole grain durum *vs.* whole grain Kamut® khorasan) and bread-making (baker's yeast *vs.* sourdough fermentation). These differences cannot be accounted to single compounds, but to a best antioxidant profile of KB and even more SKB.

Further studies are needed before drawing conclusions on the effects of whole grain Kamut® khorasan wheat in humans, but until those studies are made, these reported results might suggest a safe and convenient way of increasing antioxidant protection, and underline the importance of raw material and breadmaking process for the achievement of the most effective protection. This could be significant in the light of consumers' awareness of the important impact of food and nutrition on health and well being, which is driving food processes towards the development of new products possessing higher nutritional quality.

6. ACKNOWLEDGMENTS

The authors declare that they have no conflict of interest. This work was partially supported by grants of Kamut Enterprise of Europe and of Italian MIUR (RFO A.B.).

7. REFERENCES

1. D. Giugliano and K. Esposito: Mediterranean diet and metabolic diseases. *Curr Opin Lipidol* 19, 63-68 (2008)

2. K. Poutanen, R. Shepherd, P.R. Shewry, J.A. Delcour, I. Bjorck and J.W. Van Der Kamp: Beyond whole grain: The European HEALTHGRAIN project aims at healthier cereal foods. *Cereal Foods World* 53, 32-35 (2008)

3. J. Slavin: Why whole grains are protective: biological mechanisms. *Proc Nutr Soc* 62, 129-134 (2003)

4. K.K. Adom and R.H. Liu: Antioxidant activity of grains. *J Agric Food Chem* 50, 6182-6187 (2002)

5. R.H. Liu: Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutr* 134, 3479S-3485S (2004)

6. R.H. Liu: Whole grain phytochemicals and health. *J Cereal Sci* 46, 207-219 (2007)

7. A. Fardet, E. Rock and C. Remesy: Is the *in vitro* antioxidant potential of whole-grain cereals and cereal products well reflected *in vivo*? *J Cereal Sci* 48, 258-276 (2008)

8. G.F. Stallknecht, K.M. Gilbertson, J.E. Ranney: Alternative wheat cereals as food grains: Einkorn, emmer, spelt, kamut, and triticale. In: Progress in new crops. Ed. Janick J. *ASHS Press*, Alexandria, Virginia (1996)

9. E.K. Khlestkina, M.S. Roder, H. Grausgruber and A. Borner: A DNA fingerprinting-based taxonomic allocation of Kamut wheat. *Plant Genet Resour* 4, 172-180 (2006)

10. S.M. Abdel-Aal el, J.C. Young, I. Rabalski, P. Hucl and J. Fregeau-Reid: Identification and quantification of seed carotenoids in selected wheat species. *J Agric Food Chem* 55, 787-794 (2007)

11. O. Molberg, A.K. Uhlen, T. Jensen, N.S. Flaete, B. Fleckenstein, H. Arentz-Hansen, M. Raki, K.E. Lundin and L.M. Sollid: Mapping of gluten T-cell epitopes in the bread wheat ancestors: implications for celiac disease. *Gastroenterology* 128, 393-401 (2005)

12. M.G. Ganzle, N. Vermeulen and R.F. Vogel: Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. *Food Microbiol* 24, 128-138 (2007)

13. M.E. Guerzoni, P. Vernocchi, M. Ndagijimana, A. Gianotti and R. Lanciotti: Generation of aroma compounds in sourdough: effects of stress exposure and lactobacilli-yeasts interactions. *Food Microbiol* 24, 139-148 (2007)

14. P. Vernocchi, M. Ndagijimana, D. Serrazanetti, A. Gianotti, M. Vallicelli and M.E. Guerzoni: Influence of starch addition and dough microstructure on fermentation aroma production by yeasts and lactobacilli. *Food Chem* 108, 1217-1225 (2008)

15. K. Katina, K.H. Liukkonen, A. Kaukovirta-Norja, H. Adlercreutz, S.M. Heinonen, A.M. Lampi, J.M. Pihlava and K. Poutanen: Fermentation-induced changes in the nutritional value of native or germinated rye. *J Cereal Sci* 46, 348-355 (2007)

16. K.H. Liukkonen, K. Katina, A. Wilhelmsson, O. Myllymaki, A.M. Lampi, S. Kariluoto, V. Piironen, S.M. Heinonen, T. Nurmi, H. Adlercreutz, A. Peltoketo, J.M. Pihlava, V. Hietaniemi and K. Poutanen: Process-induced changes on bioactive compounds in whole grain rye. *Proc Nutr Soc* 62, 117-122 (2003)

17. H.W. Lopez, V. Duclos, C. Coudray, V. Krespine, C. Feillet-Coudray, A. Messager, C. Demigne and C. Remesy: Making bread with sourdough improves mineral bioavailability from reconstituted whole wheat flour in rats. *Nutrition* 19, 524-530 (2003)

18. H.K. Lichtenthaler, P. Lester and D. Roland: Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol* 148, 350-382 (1987)

19. J.L. Koontz, K.M. Phillips, K.M. Wunderlich, J. Exler, J.M. Holden, S.E. Gebhardt and D.B. Haytowitz: Comparison of total folate concentrations in foods determined by microbiological assay at several experienced U.S. commercial laboratories. *J AOAC Int* 88, 805-813 (2005)

20. S. Gueguen, B. Herbeth, G. Siest and P. Leroy: An isocratic liquid chromatographic method with diode-array detection for the simultaneous determination of alpha-tocopherol, retinol, and five carotenoids in human serum. *J Chromatogr Sci* 40, 69-76 (2002)

21. N.G. Asp, C.G. Johansson, H. Hallmer and M. Siljestrom: Rapid enzymatic assay of insoluble and soluble dietary fiber. *J Agric Food Chem* 31, 476-482 (1983)

22. M.A. Bryszewska, W. Ambroziak, N.J. Langford, M.J. Baxter, A. Colyer and D.J. Lewis: The effect of consumption of selenium enriched rye/wheat sourdough bread on the body's selenium status. *Plant Foods Hum Nutr* 62, 121-126 (2007)

23. American Association of Cereal Chemists: Approved Methods of the AACC. 10th edition. *AACC International*, St. Paul, Minnesota (2000)

24. V. Verardo, M. Bonoli, E. Marconi and M.F. Caboni: Determination of free flavan-3-ol content in barley (*Hordeum vulgare* L.) air-classified flours: comparative study of HPLC-DAD/MS and spectrophotometric determinations. *J Agric Food Chem* 56, 6944-6948 (2008)

25. S. Mukherjee, S.K. Banerjee, M. Maulik, A.K. Dinda, K.K. Talwar and S.K. Maulik: Protection against acute adriamycin-induced cardiotoxicity by garlic: role of endogenous antioxidants and inhibition of TNF-alpha expression. *BMC Pharmacol* 3, 16 (2003)

26. F. Danesi, M. Malaguti, M. Di Nunzio, M. Maranesi, P.L. Biagi and A. Bordoni: Counteraction of adriamycin-induced oxidative damage in rat heart by selenium dietary supplementation. *J Agric Food Chem* 54, 1203-1208 (2006)

27. A. Bordoni, F. Danesi, M. Malaguti, M. Di Nunzio, F. Pasqui, M. Maranesi and P.L. Biagi: Dietary Selenium for the counteraction of oxidative damage: fortified foods or supplements? *Br J Nutr* 99, 191-197 (2008)

28. A.A. Elberry, A.B. Abdel-Naim, E.A. Abdel-Sattar, A.A. Nagy, H.A. Mosli, A.M. Mohamadin and O.M. Ashour: Cranberry (*Vaccinium macrocarpon*) protects against doxorubicin-induced cardiotoxicity in rats. *Food Chem Toxicol* 48, 1178-1184 (2010)

29. B. Srdjenovic, V. Milic-Torres, N. Grujic, K. Stankov, A. Djordjevic and V. Vasovic: Antioxidant properties of fullerenol C60(OH)24 in rat kidneys, testes, and lungs treated with doxorubicin. *Toxicol Mech Methods* 20, 298-305 (2010)

30. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans: Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26, 1231-1237 (1999)

31. D. Barham and P. Trinder: An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst* 97, 142-145 (1972)

32. D. Kammerer, R. Carle and A. Schieber: Characterization of phenolic acids in black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Commun Mass Spectrom* 18, 1331-1340 (2004)

33. G. Dinelli, A. Segura Carretero, R. Di Silvestro, I. Marotti, S. Fu, S. Benedettelli, L. Ghiselli and A. Fernandez Gutierrez: Determination of phenolic compounds in modern and old varieties of durum wheat using liquid chromatography coupled with time-of-flight mass spectrometry. *J Chromatogr A* 1216, 7229-7240 (2009)

34. N. Pellegrini, M. Serafini, S. Salvatore, D. Del Rio, M. Bianchi and F. Brighenti: Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweets consumed in Italy assessed by three different *in vitro* assays. *Mol Nutr Food Res* 50, 1030-1038 (2006)

35. P. Koh-Banerjee and E.B. Rimm: Whole grain consumption and weight gain: a review of the epidemiological evidence, potential mechanisms and opportunities for future research. *Proc Nutr Soc* 62, 25-29 (2003)

36. R.G. Pickering and C.E. Pickering: The effects of reduced dietary intake upon the body and organ weights, and some clinical chemistry and haematological variates of the young Wistar rat. *Toxicol Lett* 21, 271-277 (1984)

37. M. Eddouks, M. Maghrani and J.B. Michel: Hypoglycaemic effect of *Triticum repens* P. Beauv. in normal and diabetic rats. *J Ethnopharmacol* 102, 228-232 (2005)

38. R. Razack and D.L. Seidner: Nutrition in inflammatory bowel disease. *Curr Opin Gastroenterol* 23, 400-405 (2007)

39. A.S. Hazell and R.F. Butterworth: Update of cell damage mechanisms in thiamine deficiency: focus on oxidative stress, excitotoxicity and inflammation. *Alcohol Alcohol* 44, 141-147 (2009)

40. M. Soory: Relevance of nutritional antioxidants in metabolic syndrome, ageing and cancer: potential for therapeutic targeting. *Infect Disord Drug Targets* 9, 400-414 (2009)

41. S. Tesseraud, S. Metayer Coustard, A. Collin and I. Seiliez: Role of sulfur amino acids in controlling nutrient metabolism and cell functions: implications for nutrition. *Br J Nutr* 101, 1132-1139 (2009)

42. A. Bordoni, P.L. Biagi, C. Angeloni, E. Leoncini, I. Muccinelli and S. Hrelia: Selenium supplementation can protect cultured rat cardiomyocytes from hypoxia/reoxygenation damage. *J Agric Food Chem* 51, 1736-1740 (2003)

43. A. Bordoni, P.L. Biagi, C. Angeloni, E. Leoncini, F. Danesi and S. Hrelia: Susceptibility to hypoxia/reoxygenation of aged rat cardiomyocytes and its modulation by selenium supplementation. *J Agric Food Chem* 53, 490-494 (2005)

44. M.F. Andreasen, P.A. Kroon, G. Williamson and M.T. Garcia-Conesa: Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radic Biol Med* 31, 304-314 (2001)

45. S.M. Kern, R.N. Bennett, F.A. Mellon, P.A. Kroon and M.T. Garcia-Conesa: Absorption of hydroxycinnamates in humans after high-bran cereal consumption. *J Agric Food Chem* 51, 6050-6055 (2003)

46. S. Itagaki, T. Kurokawa, C. Nakata, Y. Saito, S. Oikawa, M. Kobayashi, T. Hirano and K. Iseki: *In vitro* and *in vivo* antioxidant properties of ferulic acid: A comparative study with other natural oxidation inhibitors. *Food Chem* 114, 466-471 (2009)

47. S.Y. Ou, G.M. Jackson, X. Jiao, J. Chen, J.Z. Wu and X.S. Huang: Protection against oxidative stress in diabetic rats by wheat bran feruloyl oligosaccharides. *J Agric Food Chem* 55, 3191-3195 (2007)

48. J. Wang, B. Sun, Y. Cao and Y. Tian: Protection of wheat bran feruloyl oligosaccharides against free radical-induced oxidative damage in normal human erythrocytes. *Food Chem Toxicol* 47, 1591-1599 (2009)

49. A.R. Piergiovanni, R. Simeone, A. Pasqualone: Composition of whole and refined meals of Kamut under southern Italian conditions. In: Chemical engineering

transactions. Ed. Pierucci. Vol. 17. *AIDIC Servizi S.r.l.*, Milan, Italy (2009)

50. S.K. Xia, L. Chen and J.Q. Liang: Enriched selenium and its effects on growth and biochemical composition in *Lactobacillus bulgaricus*. *J Agric Food Chem* 55, 2413-2417 (2007)

51. H. Alkreathy, Z.A. Damanhouri, N. Ahmed, M. Slevin, S.S. Ali and A.M. Osman: Aged garlic extract protects against doxorubicin-induced cardiotoxicity in rats. *Food Chem Toxicol* 48, 951-956 (2010)

52. E. Seyedrezazadeh, A. Ostadrahimi, S. Mahboob, Y. Assadi, J. Ghaemmagami and M. Pourmogaddam: Effect of vitamin E and selenium supplementation on oxidative stress status in pulmonary tuberculosis patients. *Respirology* 13, 294-298 (2008)

53. A. Kocyigit, F. Armutcu, A. Gurel and B. Ermis: Alterations in plasma essential trace elements selenium, manganese, zinc, copper, and iron concentrations and the possible role of these elements on oxidative status in patients with childhood asthma. *Biol Trace Elem Res* 97, 31-41 (2004)

54. G. Onning, A. Berggren, M. Drevelius, B. Jeppsson, A.M. Lindberg and M.L. Johansson Hagslatt: Influence of a drink containing different antioxidants and *Lactobacillus plantarum* 299v on plasma total antioxidant capacity, selenium status and faecal microbial flora. *Int J Food Sci Nutr* 54, 281-289 (2003)

55. Y. Dotan, D. Lichtenberg and I. Pinchuk: Lipid peroxidation cannot be used as a universal criterion of oxidative stress. *Prog Lipid Res* 43, 200-227 (2004)

56. K. Christou, N. Markoulis, A.N. Moulas, C. Pastaka and K.I. Gourgoulianis: Reactive oxygen metabolites (ROMs) as an index of oxidative stress in obstructive sleep apnea patients. *Sleep Breath* 7, 105-110 (2003)

57. F. Morisco, V. Verde, V. Fogliano, A. Ritieni, R. Marmo, G. De Luise, C. Tuccillo and N. Caporaso: Oxidative status in chronic hepatitis C: the influence of antiviral therapy and prognostic value of serum hydroperoxide assay. *Free Radic Res* 38, 573-580 (2004)

58. I. Ruffini, G. Belcaro, M.R. Cesarone, G. Geroulakos, A. Di Renzo, M. Milani, L. Coen, A. Ricci, R. Brandolini, M. Dugall, P. Pomante, U. Cornelli, G. Acerbi, M. Corsi, M. Griffin, E. Ippolito and P. Bavera: Evaluation of the local effects of vitamin E (E-Mousse) on free radicals in diabetic microangiopathy: a randomized, controlled trial. *Angiology* 54, 415-421 (2003)

59. E.J. Masoro: Overview of caloric restriction and ageing. *Mech Ageing Dev* 126, 913-922 (2005)

60. Y.P. Sun, C.C. Chou and R.C. Yu: Antioxidant activity of lactic-fermented Chinese cabbage. *Food Chem* 115, 912-917 (2009)

61. A. Gibreel, J.R. Sandercock, J. Lan, L.A. Goonewardene, R.T. Zijlstra, J.M. Curtis and D.C. Bressler: Fermentation of barley by using *Saccharomyces cerevisiae*: Examination of barley as a feedstock for bioethanol production and value-added products. *Appl Environ Microbiol* 75, 1363-1372 (2009)

62. M.Y. Lin and C.L. Yen: Antioxidative ability of lactic acid bacteria. *J Agric Food Chem* 47, 1460-1466 (1999)

63. R. Doblado, H. Zielinski, M. Piskula, H. Kozlowska, R. Munoz, J. Frias and C. Vidal-Valverde: Effect of processing on the antioxidant vitamins and antioxidant capacity of *Vigna sinensis* Var. Carilla. *J Agric Food Chem* 53, 1215-1222 (2005)

64. J. Denter, H.J. Rehm and B. Bisping: Changes in the contents of fat-soluble vitamins and provitamins during tempe fermentation. *Int J Food Microbiol* 45, 129-134 (1998)

Abbreviations: ABTS: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); b.w.: body weight; CFU: colony forming unit; d.w.: dry weight; DOX: doxorubicin; GPx: glutathione peroxidase; HPLC: high performance liquid chromatography; ICPMS: inductively coupled plasma mass spectrometry; KB: Kamut® khorasan bread; n.d.: not detectable; ROM: reactive oxygen molecule; RP-HPLC: reversed phase HPLC; SCFA: short-chain fatty acids; s.d.: standard deviation; SD: standard diet; SKB: sourdough Kamut® khorasan bread; STZ: streptozotocin; TAA: total antioxidant activity; TE: trolox equivalent; TPC: total phenolic compounds; WB: wheat bread.

Key Words: Antioxidant Activity, Oxidative Stress, Sourdough, Whole Wheat Bread, Kamut® khorasan

Send correspondence to: Alessandra Bordoni, Campus di Scienze degli Alimenti - Università di Bologna, Piazza Goidanich, 60 - 47521 Cesena (FC), Italy, Tel: 0039 0547 338955, Fax: 0039 0547 382348, e-mail: alessandra.bordoni@unibo.it

http://www.bioscience.org/current/vol16.htm