# Enrichment of Antioxidant Capacity and Vitamin E in Pita made from Barley

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## Abstract

This study aimed to enhance total antioxidant and vitamin E content of pita bread, by replacing 50% of the standard baker's flour with flours milled from covered (WI2585 and Harrington) or hulless (Finniss) barley genotypes, previously shown to have high antioxidant and vitamin E levels at harvest. Pita breads were made from either 100% baker's flour (control) or 50% malt flour, whole-grain flour or flour from barley grains pearled at 10%, 15% and 20% grain weight. Antioxidant capacity and vitamin E content of flours and pitas were determined by their ability to scavenge DPPH radicals and high performance liquid chromatography (HPLC), respectively. The physical and sensory properties of the pitas were also assessed. All pitas made from either whole grain or pearled barley flour had a higher antioxidant capacity and most also had higher vitamin E content than standard pita. The antioxidant and vitamin E levels were reduced in pearled compared to whole grains, however the extent of that reduction varied among genotypes. The greatest antioxidant and vitamin E levels were found in pita made from malt flour or Finniss whole grain flour. Furthermore, sensory analysis suggested these pitas were acceptable to consumers and retained similar physical and sensory properties to those in the control pita.


**Keywords**: Barley; pearling; antioxidant capacity; vitamin E; pita bread.

**Practical application:** Bread is a staple food and providing breads which are a source of fibre and bioactive compounds has the potential to provide health benefits. Here, we show that malt flour, whole-grain flour or pearled-grain flour from covered or hulless barley with high antioxidant and vitamin E levels at harvest can be used to produce pitas with higher antioxidant and vitamin E level than standard pitas, and whose sensory properties are acceptable to consumers.

## 1. Introduction

Wheat is a staple food around the world and is consumed in many forms including flat or pan style leavened bread (Pomeranz 1987). Due to the rising world population and greater awareness of a healthy lifestyle, bread containing multi-grains, whole grain or other functional ingredients is becoming more popular among consumers (Vulicevic and others 2004). A number of previous studies have demonstrated that including barley in bread improves the natural nutritional value, by increasing levels of β-glucan, minerals and antioxidants (Newman and Newman 2006).

Antioxidants in food may have a number of important health benefits, which are primarily due to their ability to slow tissue damage by preventing the formation of free radicals, scavenging them, or by promoting their decomposition (Young and Woodside 2001). In a previous study, vitamin E, a lipid phase chain-breaking antioxidant, was found at highest levels in barley caryopses compared to wheat, oats and rye (Holasova and others 1995). We have also recently demonstrated that antioxidant capacity and vitamin E content varies substantially among different barley genotypes at harvest (Do and others 2015a); and; during storage and malting (Do and others 2015b). While health claims for barley grain have been approved by the U.S. Food and Drug Administration (USFDA 2003), there is currently no published research which has determined whether and to what extent the antioxidant capacity and vitamin E content is maintained in final food products. Thus, whether breads made from barley genotypes with high antioxidant capacity and vitamin E content at harvest can be a good dietary source of these factors is unknown.

Barley is typically polished (also known as pearling) before consumption because whitened grain is generally preferred by consumers and food manufacturers (Gong and others 2012). The process of pearling removes the hull (also known as the husk), and the bran, which is firmly attached to the inner layers of the hull, is consequently abraded (Blandino and

78 others 2015). The husk and bran, both of which are rich in antioxidant capacity and vitamin
79 E, are either discarded or utilised for animal feed (Maillard and Berset 1995). While several
80 studies have been conducted on the effect of pearling on either antioxidant capacity or
81 vitamin E content on the barley grain, little work has been performed on either of these
82 components in barley products (Ko and others 2003; Panfili and others 2008; Blandino and
83 others 2015).

84 Hulless barley does not require pearling and is preferred in food production as less
85 processing is required (Elsayed and Peter 2005); the grain contains more protein, starch and
86 total soluble fibre (Bhatty 1999); and; the grain can be added directly to food (Elsayed and
87 Peter 2005). Malt made from hulless barley is of particular interest because of the same
88 advantages (Bhatty 1996). Barley malt is also ideal for bread manufacture due to high α- and
89 β-amylase enzyme activity allowing starch to be converted to maltose which can be more
90 easily digested and also promote yeast activity (Bhatty 1999). A further advantage was
91 demonstrated in our previous study (Do and others 2015b) with an increase of antioxidant
92 capacity in malt compared to unprocessed barley.

93 Little has been published on the benefits of adding different types of barley-derived
94 materials on the antioxidant capacity and vitamin E contents of pita bread. These could
95 include flour made from the whole grain, pearled grain, or malt; and; that is derived from
96 covered or hulless barley genotypes. Although there will probably be greater antioxidant
97 capacity and vitamin E content in pita bread made with higher percentages of barley, the
98 impact on the sensory quality must be positive. The appropriate combination of sensory
99 properties together with the health benefits therefore needs to be considered (Biloukha and
100 Utermohlen 2000).

101 The objectives of this study therefore were to determine antioxidant capacity and
102 vitamin E content in pita bread supplemented with barley flour made from whole-grain,

pearled-grain or malt from covered or hulless barley genotypes with high antioxidant and vitamin E levels at harvest; and; to determine their impact on the physical and sensory properties of pita bread.

## 2. Materials and Methods

### Materials

The barley varieties used in this study (the hulless genotype Finniss and the covered genotypes WI2585 and Harrington) were previously identified as being high in antioxidant capacity and vitamin E content (Do and others 2015a). Grain from each variety was used immediately after harvest to make flour, either from whole grains (0% pearling), or with 10, 15 or 20% pearling. Flour was also prepared from malt prepared from Finniss (Do and others 2015b) and after storage at 10°C for four months. The barley varieties, provided by the University of Adelaide Barley Breeding Program, were grown from June to December 2014 as a single plot in a complete randomised design at Charlick Experimental Research Station, Strathalbyn, South Australia (35°19'46.26" S, 138°52'42.39" E). The grain was hand sieved using a 2.5 mm slotted ISO 5223 sieve as per U.S. Department of Agriculture (2013).

To pearl grain, a Satake grain tester (model TM05, Tokyo, Japan) using a procedure adapted from Takenouchi Barley Processing Company Ltd, Japan (Washington and others 2003) was set at 1150 rpm with a 36 grit size wheel and was warmed up by pearling a 180 g control sample twice for 12 min each. The removed husk weight was obtained by weighing the collected pearl dust and pearling was stopped at levels of 10, 15 and 20% (w/w) of husk removed.

Malt (eight cans, 60 g each) was obtained from Finniss using a Phoenix Automatic Micromalting System®, in accordance with the standard protocol used by the Barley Quality Laboratory at The University of Adelaide (Cozzolino and others 2014).

All samples (malt, whole grain and pearled grain) were ground to a fine powder using a UDY Cyclone Sample Mill (Udy Corporation, Boulder, CO, USA). The resultant flour was used for pita bread preparation.

**Pita preparation**

Pitas were prepared and cooked as per Bailey (2007) with some modifications. Flour (50 g), instant dry yeast (0.4%; Defiance Quality Food, Australia), salt (1.8%), sugar (1%), and water at 30°C (60-65%) were mixed using a 50 g micro-mixer (National MFG Co, Lincoln, USA) for 15 min. Control samples were made with 100% commercial baker's flour (Defiance Quality Food, Australia) while in the other samples, 50% of the flour was replaced with barley flour prepared from malt, whole grain or pearled grain as per Malcolmson and others (2011). Each dough was rounded into a ball, placed in a 75x50x32 mm mini-loafing tin and left to ferment in a sealed plastic bowl for 90 min at 30±1°C and 70% relative humidity (RH). Dough balls were then sheeted to 4 mm thick using a bench sheeter (Rondo, Germany) and then cut to 12 cm diameter using a circular pastry cutter. These dough rounds were rested in a fermentation cabinet at 30±1°C for 15 min and subsequently fried in a non-stick pan (Kambrook, Australia) for 8 min at 180°C with gentle flipping every 1 min using a wooden spatula. Cooked pitas were cooled at room temperature for 30 min and photographed (Canon, Japan) before analysis of physical parameters or sensory properties. Pita bread samples were ground with an IKA analytical mill (IKA, Malaysia) to a fine powder and stored at -80°C until vitamin E and antioxidant analysis.

**Determination of Vitamin E content and antioxidant capacity**

Tocols were extracted using saponification as per Do and others (2015a) and individual vitamin E isomers [(α, β, γ and δ-tocopherol (T) and tocotrienol (T3)] quantified

153 using HPLC (Do and others 2015a). The vitamin E content, expressed in mg of α-tocopherol-
154 equivalents (TE), was calculated using the equation: α-TE = α-T*1.0 + α-T3*0.3 + β-T*0.4 +
155 β-T3*0.05 + γ-T*0.1 + γ-T3*0.01 + δ-T*0.01 (McLaughlin and Weihrauch 1979).

156 Antioxidants were extracted using 80% ethanol and antioxidant capacity measured
157 using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as per Do and others (2015a). Antioxidant
158 activity was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g
159 fresh weight (FW) of grain.
160
161 **Physical parameters**

162 Dough height was measured after fermentation using Digimax digital calipers
163 (Camlab Limited, UK) while the extent of pocket formation (or puffing) was observed during
164 baking (full, ¾, ½ or not at all). After baking, an image of each pita was captured and the
165 thickness measured using the calipers. If the pita was fully puffed, the thickness of the upper
166 and lower layers was also measured. The upper layer was defined as the top layer of dough
167 during the resting time after sheeting and was placed first in the pan for cooking.

168 Pita firmness was determined using a compression test according to the AACC
169 approved method 74-09 (AACC, 2000) with some modifications. A food texture analyser
170 (Mecmesin Imperial 1000 Motorised Test Stand, West Sussex, England) equipped with a 100
171 N load cell was used to compress the pita with a 25 mm flat aluminium plunger up to 40%
172 maximum strain at a speed of 1.7 mm/second at 20°C. Pre-test and post-test speeds were 1.0
173 mm/second and 10.0 mm/second, respectively. The bread was laid on the texture analyser
174 platform, and the distance between the platform and the plunger set to 5 cm. Although
175 compression tests are usually conducted on crust-less high or medium volume bread, the crust
176 in the case of flat bread is very thin and difficult to remove without taking parts of the crumb.

177 For this reason, the first 25% of the analysis was discarded and firmness was defined as the
178 force at 40% strain minus the force at 25% strain (Alhajji 2011).

179 The colour of flour and pita samples was measured (Minolta Colorimeter CR-300,
180 Ramsey, NJ) and data recorded using the $L^*$ (lightness), $a^*$ (green [−a] to red [+a]) and $b^*$
181 (blue [−b] to yellow [+b]) colour system.

182

183 **Sensory analysis**

184 Sensory evaluation (University of Adelaide Human Research Ethics Committee
185 approval number H-2015-156) was conducted by 52 consumers (28 females and 24 males;
186 18-60 years old; and; students or staff of the University of Adelaide). After providing written
187 informed consent, each consumer was provided with a tray containing four samples (from
188 pita substituted with flour milled from malt, Finniss whole grain or 15% pearled WI2585 and
189 control pita made from baker's flour). All samples were coded with randomly selected three
190 digit numbers and presented together with a scorecard in a randomized order and room
191 temperature water for mouth cleansing between testing samples (Meilgaard and others 2007).
192 Consumers were asked to record the acceptability for appearance, texture, flavor and taste on
193 a 9-point hedonic scale (1 = dislike extremely; 5 = neither dislike nor like and 9 = like
194 extremely) (Shewfelt and others 2015) as well as the intensity scores for color (1 = dark and 9
195 = light), texture (1 = firm and 9 = soft) and flavor and taste (1 = none and 9 = high).

196

197 **Statistical analysis**

198 In order to determine the differences between means using the Least Significant
199 Difference (LSD) at $P<0.05$, one-way and two-way Analysis of Variance (ANOVA) was
200 performed using Genstat 14 (VSN International Ltd., Hemel Hempstead, UK). Correlation
201 analysis was performed using Microsoft Excel.

202

203 **3. Results and Discussion**

204 **Pearling removes antioxidant capacity and vitamin E content from barley grain**

205 Regardless of genotype, the levels of vitamin E and its isomers were significantly
206 higher in the flour made from whole grain than in flour made from pearled grain even in the
207 case of the lowest amount of pearling (10%) (Figure 1A). The extent of this reduction was
208 greatest for the hulless variety (Finniss), which exhibited an approximately two-fold decrease
209 for α-T and β-T3 and three-fold decrease for α-T3 and δ-T3 isomers, accounting for the
210 observation that flour made from 10% pearled grain was three-fold lower in vitamin E
211 content than the flour made from whole grain (Table 1). The levels of vitamin E and the
212 majority of isomers were further reduced in the flour made from 20% pearled grain,
213 suggesting that most isomers decreased progressively from the external to the internal layers.
214 This was especially obvious in the covered genotypes, WI2585 and Harrington, and was
215 associated with a roughly two-fold decrease in vitamin E content in the flour at 20% pearling.
216 The only two isomers which appeared to be stable during all pearling stages were β-T and γ-
217 T, however, they were only present at very low levels (<1 μg/g DW) in most samples.

218 Tocol content in the hulless Finniss variety decreased most after the first pearling step
219 while in the covered genotypes, Harrington and WI2585, tocol content decreased most after
220 the second and third pearling steps, respectively. According to Ko and others (2003), the hull
221 accounts for ~10.1% of whole grain weight whereas bran layers, which are richest in
222 tocotrienols, account for ~12.6% and germ, rich in tocopherols, accounts for ~0.6% of whole
223 grain weight. Thus, the extent of the reduction in vitamin E observed after pearling in the
224 current study indicates that the pearling levels abraded not only the hull but also the bran and
225 part of the germ but in a genotype-dependent manner. For the hulless Finniss, more bran and
226 germ might be removed with 20% pearling than for covered varieties. For WI2585, 15%

pearled grain was significantly lower in vitamin E than 10% pearled grain with further significant reductions at 20% pearling. However, for Harrington, vitamin E reduced most at the 15% pearling stage with limited further reduction at the 20% pearling stage suggesting that the thickness of the outer layers may vary between these two genotypes. Even though Ko and others (2003) reported that the relative weight of the hull, bran and germ can be influenced by growing conditions and location, all genotypes in our study were grown under the same environmental conditions, suggesting that any variation in this parameter was due to genotype.

Even though pearling reduced vitamin E content, the vitamin E content of pearled grain from the covered genotypes (WI2585 and Harrington), regardless of amount of pearling, was still significantly greater than the vitamin E content in the standard baker's flour (Figure 1A). The vitamin E content of flour made from the 10% pearled grain from Finniss and flour from malt was also significantly greater than the standard flour.

Storage had no effect on the vitamin E content in flour made from the whole grain from any of the genotypes, as expected (Do and others 2015a). Flour from Finniss whole grain was richest in vitamin E content among the samples but malt prepared from Finniss had a reduced level of vitamin E. This finding confirms our previous study that vitamin E decreases during malting because of leakage into the water during steeping and high temperature during kilning (Do and others 2015b).

Although the control sample ranked seventh out of 14 samples in terms of α-T content in the flour, its α-T3 content was lowest (2.2 µg/g DW) (Table 1) resulting in a low content of vitamin E (26.7 µg/g DW) (Figure 1A). In contrast, the highest level of α-T was found in flour made from the whole grain of Harrington and WI2585, followed by flour from the 10% pearled WI2585. The level of α-T3 was twenty to thirty times greater in flour prepared from whole grains of Finniss, Harrington and WI2585 as well as 10% pearled grain of Harrington

252 compared to standard baker's flour. Flour prepared from malt had a significantly lower α-T
253 content than control flour but was nineteen times higher in α-T3 content resulting in a high
254 vitamin E content, which was two-fold greater than the control. Although α-T has historically
255 been reported as the most efficient antioxidant (McLaughlin and Weihrauch 1979), α-T3 has
256 recently been shown to be at least three-fold more efficient as a scavenger of peroxyl radicals
257 than α-T (Packer 1995). In our previous study (Do and others 2015a), α-T3 was the main
258 vitamin E isomer in barley grain, regardless of genotype, and the correlation of α-T3 with
259 antioxidant capacity supports this observation. Storage increased content of β-T3 and γ-T3 in
260 flour prepared from whole grain of WI2585 while γ-T3 was significantly greater in Finniss
261 whole grain. However, no significant change was observed in the two main isomers, α-T and
262 α-T3, and consequently the vitamin E content in flour prepared from stored grain, regardless
263 of genotype.

264 Similar to what was observed for vitamin E content, a progressive decrease in the
265 antioxidant capacity of flour was also observed with pearling for all genotypes (Figure 2A).
266 At 10% pearling, the loss of antioxidant capacity in descending order of flour made from
267 Finniss, Harrington and WI2585 was ~48%, 23% and 3%, respectively, whereas at 15%
268 pearling the antioxidant capacity lost in those genotypes was ~52%, 38% and 15%,
269 respectively. When 20% of the grain was pearled, the highest percentage decrease of
270 antioxidant capacity was observed in flour from the hulless variety, Finniss (55%) followed
271 by Harrington (~49%) and WI2585 (28%). The decrease in antioxidant capacity for hulless
272 Finniss and covered Harrington primarily occurred with 10% pearling, suggesting that the
273 10% pearl fraction contains the majority of antioxidants, including both vitamin E (Figure
274 1A) and other phenolic compounds (Goupy and others 1999). However, we previously found
275 that vitamin E was not the main contributor to antioxidant capacity in barley (Do and others
276 2015a), and therefore the removal of other phenolic compounds by pearling is likely to have

277 the greatest impact. Total soluble phenolic content of the first fraction (10% pearling) has
278 previously been shown to be double that of the second fraction (20% pearling) in two other
279 hulless genotypes examined by Gong and others (2012). Previous studies of covered
280 genotypes have shown that *p*-coumaric acid levels increased dramatically in the outer grain
281 layers, especially in lignified husk (Salomonsson and others 1980) while ferulic acid was
282 highest in the cell walls of the aleurone layer (McNeil and others 1975).

283 While most antioxidants were removed in the first pearling stage for Finniss and
284 Harrington, this appeared to occur in the two subsequent pearling stages for WI2585, which
285 reflected the pattern observed for vitamin E. Environment affects antioxidant capacity in
286 wheat (Moore and others 2006), but does not appear to do so in barley (Peterson and Qureshi
287 1993). However, barley was only grown in one environment in this study and therefore the
288 impact of environment on antioxidant capacity requires further investigation. The differences
289 in the effect of pearling on antioxidant capacity in the different genotypes are more likely to
290 be related to genotypic variation affecting the development of the outer layers of the grain
291 and the concentration or types of antioxidant present. According to Evers and others (1999)
292 the hull amount can range between 7-25% of grain weight depending upon genotype,
293 growing conditions and grain size. Finniss is a hulless genotype and was therefore expected
294 to lose antioxidant capacity more quickly with pearling. Harrington has a loose adhering husk
295 and is highly susceptible to skinning (Menz 2010). In contrast, WI2585 has a thicker and
296 more adhering husk which might result in a higher percentage of husk per grain weight and
297 explain the differences between these genotypes in terms of the loss of antioxidant capacity in
298 flour from pearled grain. In addition, some researchers have reported that phenolic acids are
299 concentrated in the cell walls of outer layers (Maillard and Berset 1995), while others have
300 indicated that phenolic acids were mainly present in the aleurone layer and endosperm due to
301 genotypic differences (Goupy and others 1999). However, the relationship between phenolics

and which layers they are concentrated in for these genotypes remains to be established as does the availability of phenolics (bound versus free) (Lu and others 2007).

As expected, the antioxidant capacity of the control wheat sample was four-times lower than that of barley flour at the highest pearling level (20%) and ten-times lower than that of flour prepared from barley whole grains, regardless of genotype (Figure 2A). This finding is in agreement with a previous study which showed a lower antioxidant capacity in wheat grain compared to barley grain (Holasova and others 1995). Additionally, wheat pearling to produce white baker's flour has been shown to reduce antioxidant capacity linearly as the degree of pearling increased (Liyana-Pathirana and others 2006). Flour from Harrington whole grain had the highest antioxidant capacity, followed by flour from malt, flour from WI2585 whole grain and Finniss whole grain. Malt prepared from Finniss had a slightly higher antioxidant capacity compared to unprocessed Finniss, a finding which was consistent with our previous observations (Do and others 2015b). In contrast, storage reduced the antioxidant capacity by 10.5%, 11.8% and 14.5% in whole grain of WI2585, Finniss and Harrington, respectively, as per Do and others (2015a).

**Barley pitas contain higher antioxidant and vitamin E content**

The reduction of vitamin E content and antioxidant capacity after pearling in raw samples resulted in reduction of these same components in pita breads (Figure 1B and 2B). In addition, the pita breads made from barley flour or malt had a significantly lower antioxidant capacity and vitamin E content than that in the flour, however this was not the case for the pita made with 100% baker's flour. After cooking, the antioxidant capacity decreased by 41-59% and vitamin E content by 50-77%. Vitamin E content reduced by roughly three-fold for Finniss and more than four-fold for WI2585 and Harrington, suggesting that vitamin E might be more stable in Finniss during cooking. Pita cooking also caused a reduction in the content

of most isomers especially the dominant types, α-T and α-T3. Even though α-T3 contributed most to total tocol content, it was negligible in pitas made with flour from 20% pearling. The content of other vitamin E isomers was generally lower in the pitas made with flour from pearled grain (Table 1) except for β-T, however, this isomer was present at significantly lower levels than all others.

Similar to vitamin E content, the antioxidant capacity in pita made from whole-grain flour was much higher than in pita made from pearled-grain flour (Figure 2B). For Finniss, antioxidant capacity significantly decreased in pita made with flour from 10% pearled grain but did not decrease further in pitas made with higher percentage pearled grain. Since antioxidant capacity was reduced in stored barley whole grain compared to that at harvest, except in the case of Harrington, the antioxidant capacity in pitas prepared with flour milled from stored whole grains was lower than in pitas prepared from fresh whole-grain flour, except in the case of Harrington.

The flour from malt had a higher antioxidant capacity than all other flour samples, and also exhibited the lowest percentage change in antioxidant capacity during cooking. Consequently, pitas made from malt flour had the highest antioxidant capacity. Although the vitamin E content of the pitas made with flour from 20% pearled grain of Finniss and Harrington were not significantly different from that observed for the wheat bakers' flour, all barley pita samples had significantly greater antioxidant capacity. This supports their potential use as functional food products as a source of antioxidants for consumers. However, even though phenolics are probably the main contributor to antioxidant capacity in barley grains (Goupy and others 1999), which compounds are responsible for the increased antioxidant capacity in the barley pita samples still requires investigation.

The losses in vitamin E content/antioxidant capacity observed in the cooked pita bread compared to the grains and flours are not unexpected, since they are known to be

unstable, especially at high temperatures. However, at increased temperature, reducing sugars and amino acids can react to produce Maillard products such as melanoidins, which also have antioxidant capacity (Maillard and others 1996). This may explain why the antioxidant capacity was not reduced to the same extent as the vitamin E content in the pitas in the present study. The antioxidant capacity also remained higher in pita made from flour of the whole grain and malt compared with that made with flour from pearled grains. Regardless of genotype, a high correlation between antioxidant capacity of the flour and the pitas made from that flour was found (r=0.85, $p<0.05$, n=14). This indicates that selecting the material with high antioxidant capacity enriches this component in pitas. A high correlation was also observed between vitamin E content in unprocessed grains or malt and in pita (r=0.81, $p<0.05$, n=14). Therefore, barley genotypes known to have high antioxidant capacity and vitamin E content can ensure much greater quantities of these components in the end product. However, the quality of the product needs to be confirmed by evaluating the physical and sensory properties as has been commonly done in other food studies (Alu'datt and others 2014; Blandino and others 2015).

**Physical quality attributes of barley pitas**

There are no specific guidelines available to judge pita bread quality but puffing formation, ease of layer separation, crust, shape and colour are considered the most important parameters (Morad and others 1984). Similar compression values (as a measure of firmness) were found in control pita and barley pitas made with flour from Finniss (regardless of whether pearled or whole grain) and pitas made from flour from 15% or 20% pearled grain from WI2585 or Harrington (Table 2). Firmness was, however, significantly greater in pita containing flour prepared from malt; 10% pearled WI2585; 10% pearled Harrington; and

whole grain of WI2585 and Harrington, both stored and fresh. Malt pita was twice as hard than the control pita while the covered whole grain pita was three times as hard.

The thickness of the pita was greatest in the control sample (15.3 mm) and lowest in pitas made from flour of the whole grains from covered genotypes (Table 2), and was negatively correlated with firmness across all samples (r=-0.9, $p<0.05$, n=17). Only pita made with flour from 20% pearled Finniss and WI2585 had similar thickness to that of control pita. Control pita also showed better crumb pore uniformity (Figure S1) and even though the upper layer did not significantly differ between any of the samples, the lower layer was significantly greater in the control than in barley pitas (Table 2). Crust with adhering crumb was observed for all pitas except those made with flour from the covered whole grain, which seemed to only have a crust in their upper layer. This crust formation happened in the thin upper layer during puffing and consequently the pocket was not fully formed.

Pocket formation did not occur during the baking of pita breads which contained flour from whole grains or 10% pearled grains of WI2585 and Harrington. In addition, three-quarter or half pockets were observed for pita made with flour from malt, stored WI2585 whole grain, stored Harrington whole grain or 10% pearled WI2585. According to Faridi and Rubenthaler (1984), pocket formation occurs when the internal temperature reaches a point high enough to develop steam during baking, but the extent to which this occurs also depends on how many bubble cells are formed in the dough during fermentation. Fewer bubble cells were observed in dough from whole grain flour from covered genotypes, which may explain why pocket formation was reduced in these pitas. In addition, the higher water absorption of the husk may have caused lower water availability in the dough for starch to be gelatinised during baking, which would also act to inhibit pocket formation (Varrianomarston and others 1980).

The softness or loaf volume of bread in general is related to the properties of the dough (Wang and others 2002). A strong correlation between dough height and thickness/firmness in the current study (r=0.9, $p<0.05$, n=17 for both) supports this observation. The height of the control dough after fermentation was higher than all barley doughs possibly due to the lack of gluten in barley, leading to lower gluten levels and consequent difficulties in dough handling, lower loaf volume and reduced softness (Wang and others 2002). Moreover, the high content of β-glucans in barley can tightly bind water in dough, thereby reducing the availability of water to develop a gluten network and rupture the bubble cells normally formed during fermentation (Wang and others 2002). Indeed, the height of the barley doughs from covered genotypes increased with increased pearling, probably due to the removal of the glucan-rich husk.

Dough height was significantly lower than the control for pitas made from malt flour while dough from Finniss whole-grain flour rose better than dough from covered whole-grain flour, indicating the advantage of hulless whole grain genotypes in food production. The differences in texture of pita made with covered genotypes, WI2585 and Harrington whole grain, could also be attributed to the difference in their content of tannin and amylose. Tannins are known to bind with protein (Hulse 1979) and are likely to form a tannin-gluten complex which might change rheological properties while differences in amylose content may cause differences in dough stickiness and therefore differences in pasting properties (Izydorczyk and others 2008).

The use of flour prepared from stored whole grain significantly increased the dough height when compared to fresh whole grain flour, and produced better pocket formation. This is consistent with previous findings in pitas made from wheat flour (Pomeranz 1992; Suter and others 1995), which indicated that two to four months storage following harvest increased loaf volume and overall baking quality. In these previous studies, the authors

suggested that this was due to an improved balance of gluten properties, an increase in protein molecular mass and higher gas-retention capacity in baking (Pomeranz 1992) as well as improved dough strength due to oxidative polymerisation of glutenins during storage (Suter and others 1995).

Lower dough and loaf volume have also been reported in previous studies on the effect of barley inclusion on properties of pita breads, however, data were provided through sensory analysis not physical testing (Alu'datt and others 2014). Reductions in loaf volume of 27% (Ragaee and others 2011) and 65% (Gujral and others 2003) have also been reported when 20% barley flour was incorporated into western-style loaf bread. However, western-style bread differs significantly from pita bread in terms of texture and due to its lower leavening requirements, pita bread might better accommodate high fibre ingredients such as barley (Blandino and others 2015; Qarooni and others 1992).

Significant differences in the $L^*$, $a^*$ and $b^*$ values were observed between the control baker's flour and flour prepared from whole grain, pearled grain or malt as well as their respective pita breads (Table 3). Control and pearled barley flour generally had higher $L^*$ (lightness) values but lower $b^*$ (blue−yellow components) and $a^*$ (red components) when compared to the flour from whole grain and malt, and this same trend was also observed in the pita. An increase in the percentage of pearling resulted in a moderate increase in the $L^*$ but a reduction in the $b^*$ for both flour and pita. In a previous study, Sumner and others (1985) reported that removal of the outer kernel layers by pearling resulted in an increase in the $L^*$ value of the pearled grain accompanied by a reduction in the red and yellow value, due to exposure of the endosperm. Interestingly, we found that, although the control baker's flour was not as light as some of the barley flours, including the flour from 15% and 20% pearled Finniss and 20% pearled WI2585 and Harrington, the control pita bread was significantly lighter than all barley pitas (Figure S1). Differences in gelatinisation of wheat and barley

starch related to moisture content (Faridi and Rubenthaler 1984) or even differences in caramelisation of sugars that may occur during baking may account for these findings, but requires further study.

Pitas made from flour from Finniss that had been pearled at any level; WI2585 and Harrington at the highest pearling level (20%), were slightly darker than control pitas, while the Finniss whole-grain flour pitas were lighter than pitas made with covered whole-grain flour. Therefore, compared to covered genotypes, hulless Finniss is likely to provide a product which is closer in appearance to standard pita bread made from wheat baker's flour in terms of brightness (Morad and others 1984). However, more recently, consumer preference for white bread has reduced as the consumption of more healthy bread has increased (Vulicevic and others 2004). Therefore, the lower brightness of the barley pitas may not necessarily reduce their acceptance by consumers. Storage appeared to slightly reduce the whiteness of whole grain but the reduction was not statistically significant for both flour and pita.

**Sensory properties of pita bread**

On the basis of the results above, pita substituted with flour milled from malt, Finniss whole grain or 15% pearled WI2585 were chosen for sensory analysis and compared to control pita made from baker's flour. Pitas made from malt and Finniss whole grain flour were chosen because antioxidant capacity and vitamin E content were high (Figures 1B and 2B) and pocket formation was satisfactory. Among pearled-barley pitas, the pita containing the highest antioxidant and vitamin E level as well as better formation was that from 15% pearled WI2585. When comparing those samples, all pitas were rated as acceptable (>5) using hedonic scales (Table 4) even though barley pitas had lower hedonic scores than control pitas.

Pita bread containing barley flour had a lower rating for colour intensity than the control indicating they were darker. This finding agreed with the observation that malt-pita had the lowest *L** value but highest *a** value, followed by Finniss whole-grain flour-pita, 15% pearled WI2585 flour-pita and the control. The change from creamy white to brown has been previously observed when barley was added to pita (Alu'datt and others 2014).

In terms of sensory evaluation, consumer texture preference for pitas made from 15% pearled WI2585 flour and whole grain Finniss flour were similar to pitas made from baker's flour, and all were higher than for malt pitas. Firmness was highly correlated to texture liking ($r=0.96$, $p<0.05$, $n=9$) and is likely to explain the findings, since the malt pita was the firmest of the breads, due to it not being fully formed during baking. Pita made from Finniss whole grain was similar to pita from 15% pearled WI2585 in both flavour intensity and liking of taste. Given that bitter-tasting phenolic compounds and tannins are usually found in the seedcoat (Abdelghafor and others 2011), hulless grain may be more ideal for making pita. In terms of overall preference, barley pita from 15% pearled WI2585 flour was the only barley pita not significantly different to the control pita but it also was not significantly different to the other barley pita. The acceptance of all samples was contributed by the liking of appearance, texture and flavour and taste with correlations of 0.99; 0.79 and 0.41 respectively with overall liking indicating the promise for future development of products.

## Conclusions

Although antioxidant and vitamin E in barley grain was lost during pearling, those components were still richer in pita made from pearled barley compared to control pita. Pitas from malt flour, Finniss whole grain flour and 15% pearled WI2585 flour contained high antioxidant capacity and vitamin E level and had satisfactory physical properties such as

499 pocket formation. Sensory evaluation indicated that they were acceptable to consumers and
500 had potential as a functional food for the bakery industry.

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**Author contributions**: Thi Thu Dung Do conducted this study and drafted the manuscript, Beverly Muhlhausler and Amanda Box helped with interpretation of results and drafting of the manuscript, Amanda Able designed this study, interpreted results and helped with drafting of the manuscript.

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635 **Table 1. Tocopherol and tocotrienol content (µg/g DW) in flour and pita after processing**. Means±SE are shown where n=3 for all flour
636 samples, n=9 for pitas from control baker's flour, flour prepared from malt and flour from Finniss whole grain; and n=3 for the remaining pita
637 samples. Same letters (within column) or * (within row) indicates no difference between samples for individual isomers or no difference
638 between flour and pita as determined using the Least Significant Difference (LSD) (P<0.05). NS indicates there was no significant difference
639 (P>0.05). δ-T3 and δ-T were not detected.
640

| Samples | α-T | | β-T | | γ-T | | α-T3 | | β-T3 | | γ-T3 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flour | Pita | Flour | Pita | Flour | Pita | Flour | Pita | Flour | Pita | Flour | Pita |
| Control (baker's flour) | 9.1±0.3$^{f}$ | 3.0±0.1$^{a}$ | 0.4±0.0$^{*}$ | 0.4±0.0 | 2.8±0.5$^{d}$ | 0.4±0.0$^{a}$ | 2.2±0.2$^{a*}$ | 1.0±0.1$^{a}$ | 12.2±0.2$^{g}$ | 1.7±0.1$^{a}$ | 0.0±0.0$^{a*}$ | 0.0±0.0$^{a}$ |
| Malt | 7.1±0.3$^{e}$ | 5.5±0.3$^{d}$ | 0.3±0.0$^{*}$ | 0.4±0.0 | 0.6±0.2$^{bc}$ | 1.4±0.1$^{e}$ | 42.0±1.6$^{h}$ | 12.2±0.4$^{g}$ | 5.5±0.2$^{d}$ | 7.1±0.4$^{h}$ | 8.3±0.4$^{e}$ | 2.3±0.1$^{ef}$ |
| Fresh Finniss (whole grain) | 12.3±0.4$^{h}$ | 4.5±0.1$^{c}$ | 0.0±0.0$^{*}$ | 0.0±0.0 | 0.6±0.0$^{bc}$ | 1.9±0.3$^{f}$ | 63.8±2.1$^{l}$ | 11.8±0.6$^{fg}$ | 12.2±0.5$^{g}$ | 9.6±0.4$^{i}$ | 14.4±0.5$^{i}$ | 5.6±0.2$^{i}$ |
| Fresh Finniss (10% pearling) | 5.2±0.1$^{c}$ | 3.7±0.0$^{bc}$ | 0.3±0.0$^{*}$ | 0.3±0.0 | 0.4±0.1$^{ab}$ | 1.9±0.0$^{f}$ | 19.7±0.4$^{e}$ | 0.8±0.2$^{a}$ | 7.2±0.4$^{e*}$ | 7.1±0.6$^{h}$ | 4.5±0.1$^{b}$ | 1.7±0.1$^{cde}$ |
| Fresh Finniss (15% pearling) | 4.4±0.0$^{b}$ | 3.1±0.1$^{ab}$ | 0.3±0.0$^{*}$ | 0.3±0.0 | 0.0±0.0$^{a}$ | 1.2±0.2$^{de}$ | 14.8±0.1$^{c}$ | 0.4±0.0$^{a}$ | 6.6±0.0$^{e}$ | 5.2±0.2$^{fg}$ | 3.1±0.1$^{a}$ | 1.0±0.0$^{bc}$ |
| Fresh Finniss (20% pearling) | 3.6±0.1$^{a*}$ | 3.1±0.1$^{ab}$ | 0.3±0.0$^{*}$ | 0.3±0.0 | 0.0±0.0$^{a}$ | 0.8±0.0$^{abcd}$ | 11.5±0.2$^{b}$ | 0.4±0.0$^{a}$ | 6.7±0.3$^{e}$ | 5.0±0.1$^{f}$ | 2.3±0.0$^{a}$ | 0.8±0.0$^{ab}$ |
| Fresh WI2585 (whole grain) | 13.1±0.1$^{i}$ | 4.6±0.2$^{c}$ | 0.0±0.0$^{*}$ | 0.4±0.0 | 0.8±0.1$^{bc*}$ | 0.7±0.1$^{abc}$ | 44.4±1.1$^{i}$ | 6.4±0.1$^{cd}$ | 4.4±0.6$^{bc}$ | 3.5±0.5$^{bcd}$ | 10.2±1.3$^{g}$ | 2.7±0.3$^{f}$ |
| Fresh WI2585 (10% pearling) | 12.9±0.0$^{i}$ | 4.5±0.6$^{c}$ | 0.3±0.0$^{*}$ | 0.0±0.0 | 0.8±0.1$^{bc}$ | 1.2±0.4$^{de}$ | 39.3±0.5$^{g}$ | 3.3±0.8$^{b}$ | 4.2±0.5$^{ab*}$ | 4.0±0.7$^{de}$ | 9.3±1.0$^{f}$ | 2.7±0.6$^{f}$ |
| Fresh WI2585 (15% pearling) | 10.7±0.4$^{g}$ | 3.6±0.0$^{b}$ | 0.3±0.0$^{*}$ | 0.4±0.0 | 0.8±0.1$^{bc*}$ | 0.6±0.0$^{ab}$ | 29.9±0.3$^{f}$ | 3.2±0.1$^{b}$ | 3.7±0.7$^{ab*}$ | 3.1±0.1$^{bc}$ | 7.1±1.2$^{d}$ | 1.3±0.2$^{bcd}$ |
| Fresh WI2585 (20% pearling) | 6.9±0.6$^{e}$ | 3.4±0.0$^{ab}$ | 0.0±0.0$^{*}$ | 0.3±0.0 | 0.5±0.0$^{bc*}$ | 0.6±0.0$^{ab}$ | 17.6±2.7$^{d}$ | 1.6±0.3$^{ab}$ | 3.5±0.5$^{a*}$ | 3.0±0.0$^{b}$ | 4.5±0.6$^{b}$ | 1.2±0.0$^{bcd}$ |
| Fresh Harrington (whole grain) | 13.2±0.5$^{i}$ | 4.6±0.1$^{c}$ | 0.3±0.0$^{*}$ | 0.4±0.0 | 1.1±0.3$^{c*}$ | 1.1±0.0$^{cde}$ | 55.2±0.5$^{j}$ | 6.5±0.7$^{cd}$ | 9.4±0.2$^{f}$ | 7.2±0.1$^{h}$ | 18.0±0.0$^{k}$ | 5.7±0.2$^{i}$ |
| Fresh Harrington (10% pearling) | 10.5±0.3$^{g}$ | 4.1±0.2$^{bc}$ | 0.3±0.0$^{*}$ | 0.3±0.0 | 0.8±0.3$^{bc*}$ | 1.0±0.0$^{bcde}$ | 43.4±0.2$^{hi}$ | 6.9±0.8$^{cd}$ | 8.7±0.2$^{f}$ | 7.0±0.5$^{h}$ | 14.7±0.1$^{i}$ | 4.9±0.4$^{hi}$ |
| Fresh Harrington (15% pearling) | 5.9±0.3$^{d}$ | 3.2±0.1$^{ab}$ | 0.3±0.0$^{*}$ | 0.0±0.0 | 0.0±0.0$^{a}$ | 0.8±0.1$^{abcd}$ | 18.0±1.5$^{de}$ | 0.7±0.2$^{a}$ | 4.4±0.3$^{bc*}$ | 4.4±0.4$^{ef}$ | 5.9±0.5$^{c}$ | 1.9±0.2$^{def}$ |
| Fresh Harrington (20% pearling) | 5.2±0.3$^{c}$ | 2.9±0.0$^{a}$ | 0.3±0.0$^{*}$ | 0.3±0.0 | 0.0±0.0$^{a}$ | 0.7±0.1$^{abc}$ | 16.1±0.0$^{cd}$ | 0.2±0.0$^{a}$ | 5.2±0.4$^{d}$ | 3.9±0.2$^{de}$ | 4.9±0.7$^{b}$ | 0.9±0.1$^{bc}$ |
| Stored Finniss (whole grain) | 12.1±0.1$^{h}$ | 4.6±0.0$^{c}$ | 0.3±0.0$^{*}$ | 0.0±0.0 | 0.6±0.0$^{bc*}$ | 1.0±0.0$^{bcde}$ | 63.0±0.5$^{l}$ | 9.1±0.2$^{e}$ | 12.4±0.1$^{g}$ | 7.5±0.2$^{h}$ | 15.6±0.1$^{j}$ | 4.7±0.1$^{h}$ |
| Stored WI2585 (whole grain) | 13.3±0.0$^{i}$ | 4.4±0.1$^{c}$ | 0.4±0.0$^{*}$ | 0.4±0.0 | 0.9±0.0$^{c*}$ | 0.7±0.0$^{abc}$ | 43.7±0.0$^{hi}$ | 5.8±0.5$^{c}$ | 5.1±0.0$^{cd}$ | 3.8±0.1$^{cde}$ | 11.8±0.0$^{h}$ | 3.2±0.1$^{g}$ |
| Stored Harrington (whole grain) | 13.1±0.3$^{i}$ | 4.2±0.1$^{c}$ | 0.4±0.0$^{*}$ | 0.4±0.0 | 0.4±0.0$^{ab*}$ | 0.7±0.1$^{abc}$ | 56.6±1.4$^{k}$ | 7.9±0.3$^{de}$ | 8.9±0.4$^{f}$ | 5.9±0.8$^{g}$ | 18.1±0.7$^{k}$ | 5.4±0.4$^{hi}$ |
| *LSD* | 0.5 | 0.5 | NS | NS | 0.4 | 0.4 | 1.9 | 1.9 | 0.7 | 0.7 | 0.8 | 0.8 |

641 **Table 2 Instrumental texture analysis values of different pita bread**. Means±SE are
642 shown where n=3 for each sample. Same letters (within column) indicates no difference
643 between samples as determined using the Least Significant Difference (LSD) ($P$<0.05). Not
644 applicable (n/a) – not measured. NS indicates no significant difference.

| Samples | Dough height (mm) | Thickness (mm) | Upper layer thickness (mm) | Lower layer thickness (mm) | Compression (N) | Pocket formed |
|---|---|---|---|---|---|---|
| Control (baker's flour) | 35.0±0.6$^{h}$ | 15.3±0.2$^{h}$ | 5.0±0.2 | 7.8±0.1$^{d}$ | 2.8±0.4$^{a}$ | Fully |
| Malt | 28.1±0.2$^{c}$ | 9.2±0.1$^{ab}$ | n/a | n/a | 6.5±0.3$^{b}$ | 3/4 |
| Fresh Finniss (whole grain) | 29.8±0.1$^{d}$ | 12.8±1.0$^{ef}$ | 3.9±0.2 | 4.5±0.2$^{abc}$ | 4.2±0.8$^{a}$ | Fully |
| Fresh Finniss (10% pearling) | 32.0±0.2$^{f}$ | 13.6±0.2$^{efg}$ | 4.6±0.3 | 4.8±0.2$^{abc}$ | 3.5±0.0$^{a}$ | Fully |
| Fresh Finniss (15% pearling) | 33.5±0.0$^{g}$ | 13.9±0.1$^{efg}$ | 4.1±0.1 | 5.9±0.4$^{c}$ | 3.3±0.6$^{a}$ | Fully |
| Fresh Finniss (20% pearling) | 33.7±0.1$^{g}$ | 14.1±0.2$^{fgh}$ | 4.0±0.1 | 4.8±0.1$^{abc}$ | 3.3±0.4$^{a}$ | Fully |
| Fresh WI2585 (whole grain) | 27.0±0.4$^{b}$ | 8.6±0.0$^{a}$ | n/a | n/a | 8.4±1.3$^{cd}$ | No |
| Fresh WI2585 (10% pearling) | 27.9±0.1$^{c}$ | 9.3±0.1$^{ab}$ | n/a | n/a | 6.7±0.3$^{b}$ | 1/2 |
| Fresh WI2585 (15% pearling) | 30.7±0.0$^{e}$ | 13.9±0.6$^{efg}$ | 4.5±0.7 | 5.5±1.2$^{bc}$ | 4.1±0.2$^{a}$ | Fully |
| Fresh WI2585 (20% pearling) | 32.1±0.2$^{f}$ | 14.2±0.6$^{gh}$ | 4.3±0.6 | 5.9±1.0$^{c}$ | 3.2±0.2$^{a}$ | Fully |
| Fresh Harrington (whole grain) | 26.0±0.2$^{a}$ | 9.1±1.0$^{ab}$ | n/a | n/a | 8.8±0.4$^{d}$ | No |
| Fresh Harrington (10% pearling) | 27.0±0.3$^{b}$ | 10.1±0.6$^{bc}$ | n/a | n/a | 7.0±0.1$^{bc}$ | No |
| Fresh Harrington (15% pearling) | 29.3±0.2$^{d}$ | 10.7±0.2$^{c}$ | 4.1±0.5 | 4.2±0.3$^{ab}$ | 3.9±0.2$^{a}$ | Fully |
| Fresh Harrington (20% pearling) | 30.9±0.0$^{e}$ | 11.3±0.6$^{cd}$ | 4.2±0.1 | 4.1±0.2$^{ab}$ | 4.2±0.4$^{a}$ | Fully |
| Stored Finniss (whole grain) | 31.0±0.0$^{e}$ | 13.7±0.3$^{efg}$ | 4.2±0.6 | 3.7±0.4$^{a}$ | 4.0±0.7$^{a}$ | Fully |
| Stored WI2585 (whole grain) | 29.5±0.0$^{d}$ | 12.6±0.5$^{de}$ | n/a | n/a | 6.6±0.3$^{b}$ | 3/4 |
| Stored Harrington (whole grain) | 28.0±0.0$^{c}$ | 10.3±0.3$^{bc}$ | n/a | n/a | 7.1±0.4$^{bc}$ | 3/4 |
| LSD | 0.6 | 1.3 | NS | 1.6 | 1.5 | |

645

646 **Table 3. *L*, a* and b** colour values of different pita bread**. *L**, lightness component; *a**,
647 green (-*a*) to red (+*a*); *b**, blue (-*b*) to yellow (+*b*). Means±SE are shown for n=9 for each
648 sample. Same letters (within column) indicates no difference between samples for as
649 determined using the Least Significant Difference (LSD) ($P$<0.05). LSD=1.30; 0.47 and 0.67
650 for *L**, *a** and *b**, respectively.

| Samples | Flour | | | Pitas | | |
|---|---|---|---|---|---|---|
| | ***L**** | ***a**** | ***b**** | ***L**** | ***a**** | ***b**** |
| Control (Baker flour) | 94.1±0.0$^{bc}$ | 0.6±0.0$^{a}$ | 9.0±0.0$^{fgh}$ | 77.6±0.6$^{g}$ | 1.2±0.1$^{c}$ | 16.1±0.3$^{k}$ |
| Malt | 86.6±0.0$^{f}$ | 2.2±0.0$^{d}$ | 10.1±0.0$^{i}$ | 62.3±0.2$^{n}$ | 7.6±0.0$^{l}$ | 26.0±0.1$^{v}$ |
| Fresh Finniss (Whole grain) | 92.7±0.0$^{de}$ | 1.1±0.0$^{bc}$ | 8.7±0.0$^{efg}$ | 67.7±0$^{jk}$ | 4.4±0.0$^{j}$ | 20.8±0.1$^{q}$ |
| Fresh Finniss (10% pearling) | 96.0±0.0$^{a}$ | 0.6±0.0$^{a}$ | 6.0±0.0$^{bc}$ | 69.4±1.3$^{i}$ | 8.0±0.9$^{l}$ | 27.6±0.8$^{w}$ |
| Fresh Finniss (15% pearling) | 95.8±0.0$^{a}$ | 0.5±0.0$^{a}$ | 5.8±0.0$^{a}$ | 71.0±0.3$^{h}$ | 2.6±0.1$^{def}$ | 16.8±0.3$^{lm}$ |
| Fresh Finniss (20% pearling) | 96.1±0.0$^{a}$ | 0.5±0.0$^{a}$ | 5.3±0.0$^{a}$ | 71.6±0.3$^{h}$ | 2.5±0.1$^{de}$ | 17.2±0.2$^{mn}$ |
| Fresh WI2585 (Whole grain) | 91.7±0.0$^{e}$ | 0.9±0.0$^{abc}$ | 9.2±0.0$^{gh}$ | 64.1±0.4$^{m}$ | 4.5±0.0$^{j}$ | 25.0±0.3$^{u}$ |
| Fresh WI2585 (10% pearling) | 93.7±0.0$^{cd}$ | 0.8±0.0$^{abc}$ | 8.4±0.0$^{ef}$ | 67.9±0.4$^{jk}$ | 3.9±0.0$^{i}$ | 22.2±0.2$^{r}$ |
| Fresh WI2585 (15% pearling) | 94.3±0.0$^{bc}$ | 0.7±0.0$^{ab}$ | 7.4±0.0$^{d}$ | 67.3±1.8$^{kl}$ | 3.2±0.0$^{gh}$ | 19.4±0.1$^{p}$ |
| Fresh WI2585 (20% pearling) | 95.1±0.0$^{ab}$ | 0.7±0.0$^{ab}$ | 6.6±0.0$^{c}$ | 71.3±0.4$^{h}$ | 2.6±0.0$^{def}$ | 18.5±0.1$^{o}$ |
| Fresh Harrington (Whole grain) | 92.0±0.0$^{e}$ | 1.1±0.0$^{bc}$ | 10.1±0.0$^{i}$ | 59.8±0.4$^{o}$ | 4.8±0.0$^{jk}$ | 23.3±0.0$^{s}$ |
| Fresh Harrington (10% pearling) | 94.1±0.0$^{bc}$ | 0.8±0.0$^{abc}$ | 8.2±0.0$^{e}$ | 66.3±0.6$^{l}$ | 3.5±0.1$^{hi}$ | 19.8±0.5$^{p}$ |
| Fresh Harrington (15% pearling) | 94.8±0.0$^{abc}$ | 0.7±0.0$^{ab}$ | 7.5±0.0$^{d}$ | 67.3±0.6$^{kl}$ | 2.9±0.3$^{efg}$ | 18.5±0.8$^{o}$ |
| Fresh Harrington (20% pearling) | 95.5±0.0$^{ab}$ | 0.7±0.0$^{ab}$ | 6.5±0.0$^{c}$ | 68.9±0.7$^{ij}$ | 3.0±0.0$^{fg}$ | 17.7±0.2$^{n}$ |
| Stored Finniss (whole grain) | 92.6±0.0$^{de}$ | 1.1±0.0$^{bc}$ | 8.9±0.0$^{fg}$ | 67.6±0.0$^{jkl}$ | 4.4±0.0$^{j}$ | 20.9±0.0$^{q}$ |
| Stored WI2585 (whole grain) | 91.6±0.0$^{e}$ | 0.9±0.0$^{abc}$ | 9.6±0.0$^{hi}$ | 64.0±0.0$^{m}$ | 4.6±0.0$^{jk}$ | 25.9±0.0$^{v}$ |
| Stored Harrington (whole grain) | 91.4±0.0$^{e}$ | 1.2±0.0$^{c}$ | 10.7±0.0$^{j}$ | 59.20.0$^{o}$ | 5.0±0.0$^{k}$ | 24.1±0.0$^{t}$ |
| LSD | 1.3 | 0.5 | 0.7 | 1.3 | 0.5 | 0.7 |

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653 **Table 4 Sensory evaluation of pita bread from baker's flour and barley flour**. Means±SE are shown for n=52 for each sample. Same letters
654 (within column) indicates no difference between samples for individual parameter as determined using the Least Significant Difference (LSD)
655 ($P$<0.05). * indicates the intensity of parameter, from dark (1 score) to light (9 score) for colour, from firm (1 score) to soft (9 score) for texture,
656 from non (1 score) to high (9 score) for flavour and taste.

| Samples | Colour intensity* | Appearance liking | Texture intensity* | Texture liking | Flavour and taste intensity* | Flavour and taste liking | Overall |
|---|---|---|---|---|---|---|---|
| Control | 7.1±0.2$^{a}$ | 6.7±0.2$^{a}$ | 5.7±0.3$^{a}$ | 6.0±0.2$^{a}$ | 6.0±0.2$^{ab}$ | 6.2±0.2$^{a}$ | 6.3±0.2$^{a}$ |
| Malt | 3.7±0.2$^{c}$ | 5.5±0.2$^{b}$ | 3.7±0.2$^{c}$ | 5.1±0.3$^{b}$ | 6.1±0.2$^{a}$ | 6.1±0.2$^{ab}$ | 5.4±0.3$^{b}$ |
| Fresh Finniss (whole grain) | 4.8±0.2$^{b}$ | 5.6±0.2$^{b}$ | 4.8±0.3$^{b}$ | 5.7±0.3$^{ab}$ | 5.4±0.3$^{bc}$ | 5.5±0.3$^{b}$ | 5.5±0.2$^{b}$ |
| Fresh WI2585 (15% pearling) | 5.2±0.2$^{b}$ | 5.9±0.2$^{b}$ | 5.0±0.3$^{ab}$ | 6.0±0.2$^{a}$ | 5.0±0.2$^{c}$ | 5.6±0.3$^{ab}$ | 5.8±0.3$^{ab}$ |
| LSD | 0.61 | 0.63 | 0.72 | 0.69 | 0.65 | 0.69 | 0.66 |

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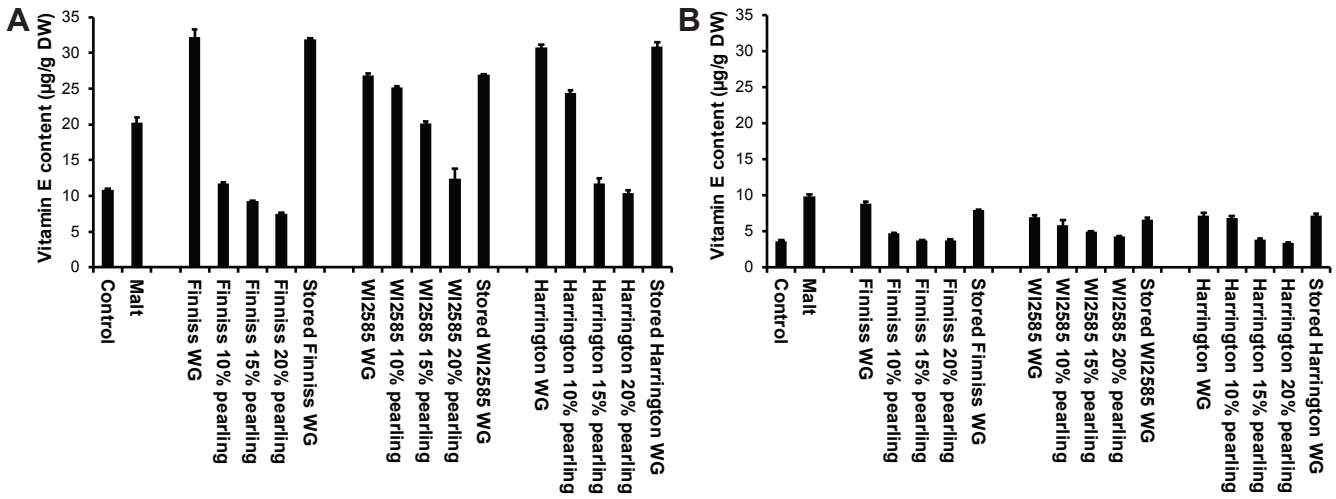


**Figure 1 Vitamin E content (µg/g FW) in flour and pita after processing.** Vitamin E is expressed in mg of α-tocopherol-equivalents (TE). Bars represent the mean ± SE. A, vitamin E content in flour before processing, n=3 for all samples. B, vitamin E content in pita after processing, n=9 for the pita from baker's flour, malt flour and Finniss whole grain flour, n=3 for the rest of pita samples. Difference between samples as determined using the Least Significant Difference (LSD) ($P<0.05$), $LSD_{sample.time}$ = 0.9. WG indicates whole grain.

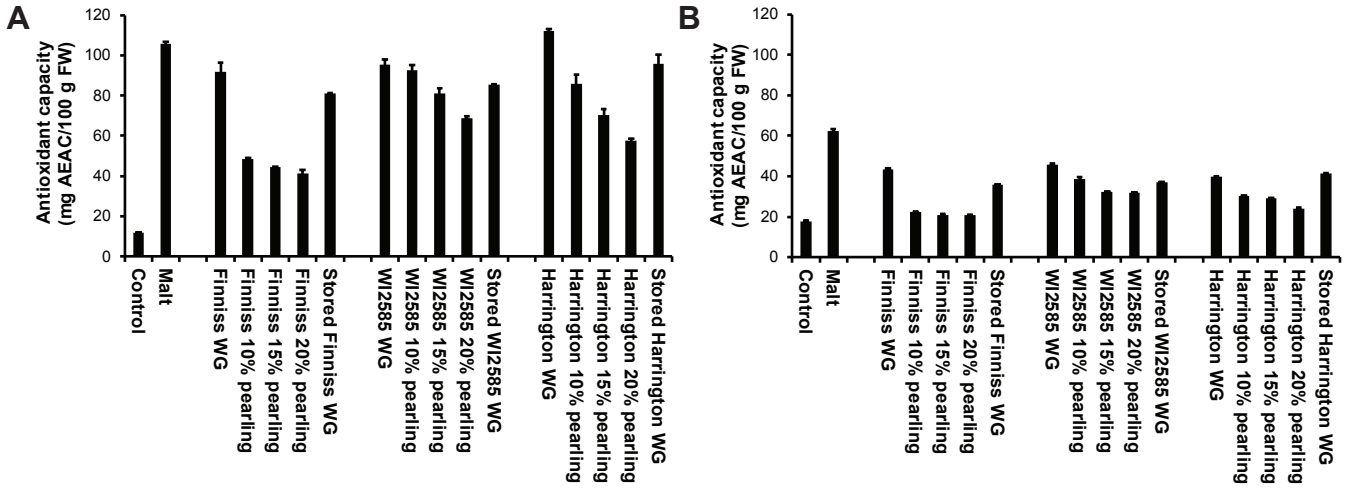


**Figure 2 Antioxidant capacity in flour and pita after processing.** Antioxidant capacity is expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g of fresh weight (FW) of grain. Bars represent the mean ± SE. A, antioxidant capacity in flour before processing, n=3 for all samples. B, antioxidant capacity in pita after processing, n=9 for pitas from baker's flour, malt flour and Finniss whole grain flour, n=3 for the rest of pita samples. Difference between samples as determined using the Least Significant Difference (LSD) ($P<0.05$), $LSD_{sample.time}$ = 2.8. WG indicates whole grain.


**Figure S1 Image of pita bread made from baker's flour and different barley flour.** A and B; pitas from baker's flour and malt flour; C, D, E, F, G; pitas from fresh Finniss whole grain flour, fresh Finniss flour at 10% pearling, fresh Finniss flour at 15% pearling, fresh Finniss flour at 20% pearling and stored Finniss whole grain flour; H, I, J, K, L; pitas from fresh WI2585 whole grain flour, fresh WI2585 flour at 10% pearling, fresh WI2585 flour at 15% pearling, fresh WI2585 flour at 20% pearling and stored WI2585 whole grain flour; M, N, O, P, Q; pitas from fresh Harrington whole grain flour, fresh Harrington flour at 10% pearling, fresh Harrington flour at 15% pearling, fresh Harrington flour at 20% pearling and stored Harrington whole grain flour.