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Antioxidant properties and sensory profiles of breads containing barley flour

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Abstract

Breads were made by replacing 40% of wheat flour with barley flour. The incorporation of barley increased the antioxidant properties of the breads compared to the control bread. Furthermore, these properties proved to be dependent on the variety of barley as well as the extraction rate of the flour. The amount of free phenolics (TPC-S) decreased during the baking process, while the amount of bound phenolics increased (TPC-IS). At the same time, the measured antioxidant activities (FRAP-S and FRAP-IS) were relatively stable during the baking process. A sensory evaluation showed differences in sensory attributes, depending on the barley variety, and there was a good consistency between the sensory evaluation and the amount of phenolics. The present study showed that utilization of barley in breads has a beneficial health potential. However this will largely depend on the barley variety.

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1. Introduction

Even though barley has been traditionally used as a food grain, it is not perceived as such an important grain today. If at all associated with food, this is most likely to be porridge, and it is certainly not associated with bread. Barley is more associated with the beverage industry (beer), malt, and especially animal feed. However, there is an increase in new food products with barley, including breads, mainly due to the content of health-related bioactive components in barley (Charalampopoulos, Wang, Pandiella, & Webb, 2002; Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). Barley is mostly known for its high amount of dietary fibre, but it also contains other important compounds, such as phenolic compounds often referred to as antioxidants.

Antioxidants in cereals exist as easily extractable compounds (free) and as less extractable types (bound) since the latter components are covalently linked to macromolecules such as arabinoxylans. In barley most of the free phenolics are flavanols and tocopherols, whereas the bound phenolics are mainly phenolic acids (ferulic acid and p-coumaric acid) (Holtekjølen, Kinitz, & Knutsen, 2006). All of these are known to have antioxidant activity and therefore, possibly health benefits (Andreasen, Landbo, Christensen, Hansen, & Meyer, 2001; Beecher, 2004). It is at present claimed that grains (e.g. barley) contain more antioxidants than previously thought due to a relatively high amount of bound components (Perez-Jimenez & Saura-Calixto, 2005). Cereals are therefore a potentially good source of natural antioxidants (Manach et al., 2004).

In addition to the possible health benefits associated with phytochemicals, these compounds have important functional properties. Firstly, phytochemicals in grains contribute to product quality in terms of colour, flavour, and texture. The phenolic acids and the flavanol polymers are perceived as sour, bitter and astringent (Dimberg,

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Molteberg, Solheim, & Frolich, 1996; Lesschaeve & Noble, 2005). Secondly, they also influence bread quality by interfering with the dough formation (Okada, Negishi, & Nagao, 1987; Piber & Koehler, 2005; Wang, van Vliet, & Hamer, 2004).

The interest in barley has increased due to its many beneficial constituents, and there is a desire to increase the amount of barley in the human diet. This can be achieved by incorporating barley in bread formulas. As far as we know, there have not been many (if any) studies on the effect of processing on the antioxidant property of breads with a fairly high amount of barley incorporated. Usually, added ingredients, acting as antioxidants, have been studied. This present research determines the antioxidant properties of breads with incorporation of different barley flour, and investigates the effect of baking and storage on the antioxidant property.

2. Materials and methods

2.1. Samples

Commercial stone-milled barley flours from three different hulled varieties, with different starch types, were used in this study. These were a normal starch variety (Tyra), a waxy starch variety (Cindy) and a high amylose starch variety (STS 2-11). Their extraction rates varied, depending on the barley variety. Tyra had the highest with 87.5%, followed by STS 2-11 (87.0%), while Cindy had the lowest, with 82.8%. Additionally, commercial white flour (Regal wheat baking flour, 78% extraction rate) and whole wheat flour (Regal wheat baking flour, 100% extraction rate) both from Läntmannen Mills (Oslo, Norway) were used. Both wheat flours had added ascorbic acid (3 g/kg flour). Fat (margarine, Pals prima) was purchased from Pals (Billingstad, Norway), while the dry yeast (saf-instant) was from S.I.LeSaffre (France).

2.2. Baking experiments

The baking procedure included mixing time, of 8 min at low speed (63 rpm), and a proving time of 60 min at 32 °C (70% moisture). Baking started at 270 °C with 30 s of steam injection, followed by a rapid lowering of the temperature to 220 °C. Total baking time was 20 min. The formulation included per dry weight, 60.00% wheat baking flour, 40.00% barley flours or whole wheat baking flour, 1.25% salt, 3.50% fat and 1.00% dry yeast. The water level added was optimized by using a Farinograph. Each dough gave seven breads of 850 g. Four different bread types were baked, three with incorporation of 40% barley flours of the three different varieties, while one was mixed with whole wheat baking flour (fine) instead of barley as a control. Both pan and hearth breads were baked to study possible differences. The baking experiment was randomized with replicates each day as well as over different days. The breads were packed in a "see through" plastic bag and stored at room temperature from 0 (fresh), 1 day and 2 days before being frozen or used in the sensory evaluation.

2.3. Pretreatments and extraction of phenolic compounds

Before analyses, the breads were thawed for 8 h. A representative sample was obtained by pooling the centre part (5 cm) and half of one end (2 cm), and homogenized with a Braun food processor (3×5 s and 1×10 s), then freezedried and ground in a Retsch centrifugal mill (Model ZM1; Retsch GmbH, Haan, Germany) with a 0.5 mm sieve before being stored cold prior to analysis.

Since grains contain both extractable (free) and covalently linked (bound) phenolics, a successive extraction was preformed in combination with a hydrolytic step. To extract the free compounds, 10 ml of cold (4 °C) 60% acetone were added to 200 mg of sample and the mixture subjected to shaking at ambient temperature for 10 min. After centrifugation (2800 rpm, 10 min) at ambient temperature, the supernatant was collected and this was referred to as the soluble or free fraction. The residual precipitate was subjected to alkali treatment (2 M NaOH, 10 ml) overnight (18 h) to release the esterified (bound) components. The suspension was neutralized and further acidified to pH 1.45-1.55 with 6 M HCl. Released bound phenolic compounds were extracted with ethyl acetate $(4 \times 10 \text{ ml},$ 10 min shaking), concentrated to dryness (Savant Speed-Vac Concentrator, SPD131DDA), and resolved in 10 ml of DMSO. This was referred to as the insoluble or bound fraction. To prevent unwanted gelatinization during this procedure, only a limited amount of sample was used (200 mg).

2.4. Determination of total phenolic compounds by the Folin– Ciocalteu method

Both the free and bound phenolic extracts were analyzed for total phenolics (TPC), as estimated by the Folin–Ciocalteu (FC) procedure (Kahkonen et al., 1999). FC reagent (1.0 ml) was added to 200 μ l of sample solution. After 2 min, 800 μ l of Na₂CO₃ were added before incubation (1 h) in darkness at room temperature. The absorbance was read at 765 nm. A standard stock solution was prepared by dissolving 50 mg of gallic acid in 5.0 ml of ethanol and diluting to 100 ml with water. The total phenolic contents are expressed as gallic acid equivalents (GAE) (mg GAE/100 g dry material). All analyses were performed in duplicate. The relative standard deviation varied between 0.2% and 9.3%.

2.5. Antioxidant activity determination by FRAP

Both the free and bound phenolic extracts were measured for antioxidant activity by FRAP (ferric reducing/ antioxidant power) according to (Benzie & Strain, 1999); 2.4 ml of TPTZ reagent (ferric 2,4,6-tripyridyl-s-triazine) was mixed with 0.1 ml of sample extract. After 1 h at room temperature, the absorbance was read at 593 nm. The antioxidant capacity (FRAP) was expressed as Fe^{3+} equivalents (mmol $Fe^{3+}/100$ g dry material). All analyses were performed in duplicate, with a relative standard deviation of 0.2–10.9%.

2.6. Sensory evaluation

The sensory evaluation was carried out by a panel of 10 well trained subjects at Matforsk AS, Norway. Each member had a minimum of five years of experience in sensory evaluation, using descriptive analysis on various kinds of foods and beverages. The panellists were selected and trained according to recommendations in ISO 8586-1 (1993) and a modified quantitative descriptive method, as described in ISO 13299 (2003). The sensory laboratory was designed according to guidelines in ISO 8589 (1988) with separate booths and electronic registration of data (CSA, Compusense Five, Version 3.80, Canada, 1999). The panel was, prior to the assessment, trained on various samples of bread, with and without barley as ingredient. The assessors developed a list of attributes for the project and agreed on a list of 17 attributes for the profiling and on the definition of each attribute. The breads were evaluated for intensity of each attribute, as fresh and after storage at room temperature for 1 day as well as 2 days. The samples were sliced into equal sizes before serving to the assessors on coded plates. The samples were analysed in duplicate at room temperature in a randomized order according to sample, replicate and assessor. For neutralization of the taste organ, the panellists were required to rinse the mouth with lukewarm water and unsalted crackers between samples. The panellists recorded their results at individual speed on a 15 cm non-structured continuous scale with the left side of the scale corresponding to the lowest intensity and the right side corresponding to the highest intensity. The computer transformed the responses into numbers between 1 = low intensity and 9 = highintensity.

2.7. Data analysis

The relationships between the different *x*-variables (barley variety, storage, pan- and hearth-baked breads and the

antioxidant properties (FRAP and TPC) (y-variables) were studied by partial least squares regression (PLSR) (Martens & Martens, 2001), using the Unscrambler software package (Version 9.2.0; CAMO A/S, Trondheim, Norway). A cross-validation, combined with a modified Jackknifing procedure (Martens uncertainty test), was used to identify significant x-variables for the prediction of y-variables (Martens & Martens, 2000, 2001). Analysis of variance and significant differences among means were tested by one-way ANOVA, using Minitab (version 14.2; Minitab Inc., State College, PA). Significant differences were declared at p < 0.05. A simple correlation (Pearson correlation) was also conducted using Minitab.

3. Results and discussion

3.1. Antioxidant properties of the flour mixtures before baking process

The antioxidant properties were investigated in the flour mixtures before the baking process. The FRAP assay was selected in order to test the total antioxidant power due its simplicity and robustness (Prior, Wu, & Schaich, 2005). In order to determine slowly reacting polyphenols, the reaction time in the assay was set as long as 1 h. Furthermore, in our laboratory, a long reaction time has been shown to give the highest correlation with the analogous (2, 2-diphenyl-1-picylhydrazyl) (DPPH) assay (Aaby, Hvattum, & Skrede, 2004). It should be noted that FRAP does not measure thiol-based antioxidants, such as glutathione (Prior et al., 2005). However, this assay is used in the present work for a relative comparison of breads originating from very similar raw materials.

The data showed clear differences between the different flour mixtures, as seen for both the TPC and the FRAP values (Table 1). Thus, the antioxidant properties varied according to the two different cereals (barley and wheat), as well as between the different barley varieties themselves. There were significantly more free phenolics than bound phenolics for all the flour mixtures, as estimated by the total phenolic test (TPC) (Table 1). For these cereals, the soluble fraction (free phenolics) will contain mostly flavanols and tocopherols, while the insoluble fraction (bound phenolics) includes mainly phenolic acids. Flavonoids and phenolic acids contribute to total phenolics in wheat

Table 1

The phenolic contents (TPC) and antioxidant activity (FRAP) of the free (S), and bound (IS) fractions obtained from the different wheat-barley mixture (60/40) and the Control before the baking process

Samples	TPC-S	TPC-IS	TPC-T	FRAP-S	FRAP-IS	FRAP-T
Wheat/Tyra (normal starch)	167 ^a	59.7 ^a	227	1.8 ^a	1.2 ^a	3.0
Wheat/Cindy (waxy starch)	165 ^a	53.2 ^{ac}	218	1.9 ^a	1.1 ^b	3.0
Wheat/STS 2-11 (high amylose starch)	83.2 ^b	10.8 ^b	94.0	0.5 ^b	$0.4^{\rm c}$	0.9
Wheat/whole wheat (Control)	96.2 ^c	46.3°	143	0.4 ^c	1.1 ^b	1.5

Different superscripts (a, b, c) in the same column are significantly different at p < 0.05. The total (T) is the sum of (S) and (IS). TPC are expressed as gallic acid equivalents (GAE) (mg GAE/100 g dry material), while the antioxidant capacity (FRAP) is expressed as Fe³⁺ equivalents (mmol Fe³⁺/100 g dry material).

(Adom & Liu, 2002), while barley also has a significant amount of proanthocyanidins (PAs) (Holtekjølen et al., 2006). Thus, in regard to the soluble fraction (S), barley will have a higher contribution from PAs than will wheat. Tocols (tocotrienols and tocopherols) and a small amount of phenolic acids will also be found in this fraction (Mattila, Pihlava, & Hellstrom, 2005).

The estimated TPC and FRAP in the free phenolic fraction varied according to type of sample, and the flour mixtures were collected into two different groups (Table 1). The mixtures with Tyra and Cindy (hereafter referred to only as Tyra and Cindy) had similar and significantly higher antioxidant properties (TPC-S and FRAP-S values) than had the two other flour mixtures. However, even if the Control mixture and the STS 2-11 mixture (referred to as Control and STS 2-11, respectively) seemed similar, the Control contained significantly higher TPC-S levels, but lower FRAP-S values than did STS 2-11.

The STS 2-11 also differed from the other flour mixtures. with low values of both FRAP and TPC for the insoluble fraction (IS), containing the bound phenolics (Table 1). However, the Control had levels of TPC-IS and FRAP-IS similar to Tyra and Cindy. The incorporation of whole wheat in the Control increased the amount of phenolic acids, which are the major phenolics in the wheat bran. In wheat, bound phenolics are reported to be up to 5-fold higher than the free phenolic content (Adom, Sorrells, & Liu, 2003; Kim, Tsao, Yang, & Cui, 2006) and can thereby explain the high amount of bound TPC and antioxidant activity in the Control. Some PAs are also reported in wheat bran (McCallum & Walker, 1990) though in significantly lower amounts than in barley. Of the three flour mixtures with high amounts of bound phenolics, Tyra had significantly higher levels of TPC than had the Control, but not than Cindy (Table 1). Also, Cindy and the Control had significantly lower FRAP-IS values than had Tyra.

When combining the free and the bound phenolics for the flour mixtures, the total amount of phenolics (TPC-T) and total antioxidant activity (FRAP-T) were highest in Tyra and in Cindy. These two barley varieties contained high amounts of both free and bound phenolics. STS 2-11, on the other hand, contained the lowest, and the Control had higher values than STS 2-11, but lower than Tyra and Cindy. Also interestingly, the mixture containing STS 2-11 had similar FRAP values for both the free (S) and the bound (IS) fractions, even though the amounts of TPC-IS were significantly lower than TPC-S. Thus, the amounts of phenolics in these two fractions were significantly different, but the antioxidant activities were not. The reason for this is not clear, but it might indicate that most of the free phenolics (TPC-S) in STS 2-11 were either less active (or artificial antioxidants) and/or they could be inhibited by other factors or were simply already oxidized. Also, even if the barley flours used had a lower extraction rate than the Control (100% extraction rate of the whole wheat), the barley contained a higher amount of TPC-S than did the Control (except STS 2-11). This is confirmed in other studies (results not shown).

3.2. Antioxidant properties of barley bread (after baking process)

Since the flour mixtures and the corresponding bread samples were prepared similarly, possible further deviations of antioxidant properties between these samples were most likely a result of the baking process. These results were also considered comparable since the contribution from the other ingredients in the bread formula was considered negligible on a dry weight basis.

During the baking process, TPC-S decreased by up to 23.5% with respect to the flour mixtures of all the breads (Fig. 1). The FRAP-S also decreased during the baking process for the breads containing barley, but not for the Control (Fig. 1). Barley contains more PAs than does wheat, and these might decrease by degradation as a consequence of the heat/thermal process during baking. However, PAs are also reported to complex with carbohydrate and protein fractions (McCallum & Walker, 1990), making them less extractable. They can also be modified by active oxidative enzymes (i.e., polyphenol oxidase) (Quinde-Axtell & Baik, 2006; Quinde, Ullrich, & Baik, 2004) or oxidized by available O_2 . Further, these compounds can complex with metal ions (ferric iron or copper) (McCallum & Walker, 1990; McDonald, Mila, & Scalbert, 1996), which is likely to interfere with the TPC estimation. Also, it is reported that, during the caramelization and breakdown of sugars (especially pentosans, notably arabinoxylans) in wheat, the furfural derivatives formed may undergo condensation with PAs during baking (McCallum & Walker, 1990). This can partly explain the decrease in free phenolics during the baking process.

In contrast to the free phenolics, the amount of bound TPC increased significantly during the baking process compared to the flour mixtures, with an increase of up to 6 fold (Fig. 1). The highest increase was seen in the STS 2-11, while the two other barleys were similar. Interestingly, even if the amount of TPC-IS increased significantly during the baking process, FRAP-IS was relatively stable/constant (Fig. 1). Thus, either the TPC method overestimated the amount of TPC-IS, or the baking process produced artificial phenolics that were extracted in the insoluble fraction. In the literature, the increase in TPC is often addressed as side effects of the baking process. The formation of heatinduced compounds from the Maillard reaction is reported as a possible contribution to the increased TPC. These compounds are also reported to have antioxidant activity (Borrelli et al., 2003), but this was not evident in our results. The antioxidant activity was rather constant during the baking process. However, Borrelli et al. (2003) used another methodology (ABTS method) to study the antioxidant activity. The different methods might explain these differences. Since different extraction methods (solvents), as well as different measurement methods, are used to

determined total phenolic compounds, as well as antioxidant activity, in the literature, direct comparison with other reported data is difficult. However, baking is reported to increase the TPC slightly (Gelinas & McKinnon, 2006), while others have claimed that phenolic compounds are destroyed during baking (Leenhardt et al., 2006). Phenolics are very unstable and reactive compounds (Cheynier, 2005), and certainly some degradation of phenolics will occur due to heat and oxidation during the baking process. Also, it is important to remember that both the TPC and FRAP methods are unspecific methods, and therefore a part of the reported values might be due to compounds that interfere with the estimation (false positives). The TPC measurement is, in fact, not specific to phenolic compounds, and many non-phenolic components can thereby interfere with the estimation of total phenolic compounds (TPC). One such interfering substance is ascorbic acid (Stratil, Klejdus, & Kuban, 2007), an additive in commercial wheat baking flours (3 g/kg). Also, saccharides, phytic acids and amino acids can interfere with these tests (Perez-Jimenez & Saura-Calixto, 2005). Further, as mentioned above, Maillard-type reaction products are involved to some extent in the estimation of phenolic compounds (Samaras, Camburn, Chandra, Gordon, & Ames, 2005). This is due to the heat-induced products (reductones and melanoidins) from the Maillard reaction, but also polyphenolic oxidation products and caramelization products can influence/affect the TPC estimation. Some of these are reported to possess antioxidant activity, while others might act as false positives in the estimations.

It is not clear whether the observed differences in antioxidant properties (before and after the baking) process in this study were due to the fermentation, kneading or the baking. However, losses of antioxidants during dough mixing, kneading and baking are reported (Leenhardt et al., 2006). The addition of water will initiate enzyme activities,



Fig. 1. Antioxidant properties (FRAP and TPC) of the different fractions (S = soluble or free, IS = insoluble or bound, T = total (S + IS)) of the different flour mixtures and the different breads after the baking process and during storage.

while a substantial incorporation of oxygen occurs during the initial dough mixing and the remoulding into smaller pieces.

In total, the breads made of Tyra and Cindy contained the highest amounts of phenolics, and had the highest antioxidant activities (i.e., showed the highest antioxidant power). The Control bread contained less TPC, in general, than did Tyra and Cindy, but more than STS 2-11. Total phenolic contents (TPC-T) (free + bound) of the breads followed the same concentration trend as did the bound phenolics, due to the larger contribution from the bound phenolics.

3.3. Influence of variety, storage and baking procedure on the antioxidant properties

To study the influence of different factors on the antioxidant properties, a multi statistical method was used (Fig. 2). The bi-plot shows the influence of variety, storage and baking (pan bread or hearth bread) on the antioxidant properties (antioxidant activity (FRAP) and amount of phenolics (TPC). Thus, the PLSR-plot shows how the variability in the antioxidant properties relates to and is explained by differences in variety type, storage and baking procedure. A total of 96% of the variability in the antioxidant properties is explained by PC1 and PC2, where PC1 explains 79% and PC2 17%. PC1 is spanned by the different varieties rather than by effect of storage (0, 1, 2) or baking procedure (p and h). Thus, the latter two have less influence on the antioxidant properties. Even if storage seemed to change the antioxidant properties slightly, these changes were in general not significant compared to the fresh breads. Also, the differences relating to pan and hearth breads were not consistent. In some cases, the hearth bread showed higher levels of TPC than did the pan breads, which most likely is related to the higher share of Maillard reactions in the former. However, no clear difference was found between the pan and the hearth breads. The antioxidant properties of breads are clearly dependent on the type of barley variety and the extraction rate of the flour, with the largest effect related to the different barley varieties. Other factors, e.g., storage and baking procedure (pan or hearth bread), were less significant. Also, there seemed to be a good consistency between the content of FRAP and TPC.

Tyra and Cindy cluster to the right in the PLSR-plot (Fig. 2), corresponding to high levels of both FRAP and TPC, while STS 2-11 is placed to the left, due to its low levels. The Control had higher levels than had STS 2-11, especially in TPC-IS levels, but less than Cindy and Tyra most likely due to the incorporation of the whole wheat and especially the bran part. The bran is known to contain a lot of bound phenolics. The Control is therefore located more in the upper middle in the PLSR-plot.

3.4. Sensory evaluation

A sensory evaluation was conducted on the breads to study possible effects on the sensory profile of each type of bread. The sensory evaluation showed clear sensory differences between the breads baked with the different barley varieties compared to the Control (Fig. 3). The higher the score in the spider diagram, the more intense is this attribute of the breads. Of the three different breads with incorporation of barley, Tyra differed from the other two in sensory profile. Tyra had a more intense flavour, as well as odour,



Fig. 2. PLSR biplot showing the influence of variety, pan and hearth baking, and storage on the antioxidant properties of the breads (amounts of free and bound phenolics (TPC-S, TPC-IS) and antioxidant activity (FRAP-S, FRAP-IS). (Co = Control, Ci = Cindy, S = STS 2-11, T = Tyra, p = pan-baked (green), h = hearth-baked (red), 0 = fresh, 1 = 1 day storage, 2 = 2 days storage). (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)



Different breads

Fig. 3. Spider web diagram of the sensory evaluation of the different breads with incorporation of different barley varieties compared to the Control. * Shows the significant levels (** = p < 0.05, *** = p < 0.01).



Fig. 4. Spider web diagram of the sensory evaluation of the different breads and the influence of storage. * Shows the significant levels (** = p < 0.05, *** = p < 0.01).

and showed the highest score for bitterness and off-odour and off-flavour, followed by Cindy of the different barley varieties. Since bitterness and off-flavours are often perceived as negative, it is likely that Tyra would have a lower acceptance among consumers than would the other barley flours. Tyra had the highest amount of phenolics (as seen from the results above). This corresponds well to the sensory evaluation, since both PAs and phenolic acids are reported to have a bitter and astringent taste (Dimberg et al., 1996; Lesschaeve & Noble, 2005). In regard to the other barley breads, STS 2-11 in general scored the lowest values (lowest intensity) of the attributes of the profiling, and was less bitter with a minor raw odour and flavour. This also corresponds well to the T-P, since STS 2-11 contained significantly lower amounts than did the other barley breads. For texture properties, juiciness and stickiness differed the most. The bread containing Cindy had the highest intensity in relation to juiciness.

Storage affected odour and flavour as well as texture of the different breads (Fig. 4). The acidity decreased significantly with storage, as did brittleness and juiciness. The bitterness, raw- and off-flavour and odour increased.

4. Conclusion

The antioxidant properties varied according to the two different cereals (barley and wheat), as well as between the different barley varieties themselves. Tyra and Cindy showed the highest antioxidant power, and the antioxidant properties of breads were clearly dependent on the type of barley variety. Thus, the largest effect on the antioxidant properties was related to the different barley varieties. Other factors, e.g., storage and baking procedure (pan or hearth bread), were less significant. The bread baked with Tyra flour contained the highest amount of phenolics and differed from the other barley breads by its more intense flavour, as well as odour. The Tyra bread showed the highest score for bitterness, off-odour and off-flavour. Also, the sensory evaluation corresponded well with the content of phenolics.

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