PROJECT REPORT 321

THE FOLATE CONTENT OF MALTED PRODUCTS: STRATEGIES FOR IMPROVEMENT

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by

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ABSTRACT

Functional, health-enhancing foods are a hot topic. Consumers are avidly searching for foods that are natural sources of nutrients and vitamins so that they can live healthier lives. While it's fairly common knowledge that cereals are a good source of vitamins, it's often not realised that malted cereals are a much better source. Malting, or germination, is in fact an ancient technique for increasing the nutritional value of seeds and it is a tradition that the maltster continues to this day.

From a scientific standpoint, we can generalise and say that during germination the growing seedling makes vitamins. However, more specific information on this topic is not available and questions such as how malting conditions or seed variety may affect this process remain something of a mystery. The aim of this project was therefore to investigate how conditions from the field to the maltings can affect this process of vitamin enhancement during malting. For this project just one vitamin, folate (or B9) was selected for study, since it is one of the vitamins most likely to be lacking in Western diets, and foods that are naturally rich in this vitamin are of particular interest.

A survey of commercial malt samples revealed that folate contents in the range 2-4 mg folate/ kg, for these products were typical. This level is 3-4 fold higher than the levels of folate in unmalted cereals. The highest folate values were measured in high diastatic potential malts (average 4 mg folate/ kg), and also in the maltings co-product roots - which contained folate levels up to 10 times that of commercial malts! This suggested that both high diastatic potential malts and roots might be of interest to the functional food market, and that there is an opportunity for the malting industry to consider the 'added value' that folate content makes to these products. Since roots are a co-product, the financial benefits of this strategy could be significant.

The project also looked at the factors that affect the increase of folate on malting. In the field, higher application of nitrogen was linked to higher folate levels in the malt, whereas seedrate and fungicide application were not influential. Also, there was a genetic influence on this process in that some varieties produced more folate on malting than others. In our pilot maltings, small-scale work also suggested that the extent of germination was an influential factor on folate content.

In summary, in the current climate of interest in health foods, the malting process has a lot to offer in the development of high value products. Malt itself has been shown by this project to be of high nutritional value. In addition, by selection of barley variety, growing and malting conditions, there is scope for the UK cereal industry to develop novel products for the functional foods market.

SUMMARY

Introduction

The demand for health-enhancing foods is growing. One of the vitamins most likely to be lacking in Western diets is folate (vitamin B9), and so foods that are naturally rich in folate are of interest to the consumer. Although cereals contain folate, malted cereals are a much better source. This is because during the malting process folate is made by the growing seedling. How this synthesis of folate is influenced by germination (i.e. malting) conditions is not known. The aim of this project was to determine which factors affect the levels of folate in malted products. This included looking at the influence of variety and growth conditions as well as malting and kilning conditions on folate content. The project also included a comparison of a range of commercially available brewing and non-brewing related malted products. With this information, strategies that the farmers and maltsters may use to increase the folate content of their products can be developed. In addition, the project provides some ideas for how new products can be developed for the food industry. Such information might in the future be used to provide a marketing advantage for UK malts.

Methods

Malting.
Malting was carried out at the BRi pilot facility. All barley samples were malted under identical conditions at the 300g scale. Samples were steeped for 8 hours, given a 16 hour air rest, then steeped again for 24 hours. The steeping liquor was then removed and germination was for 4 days at 16°C. After malting samples were either oven dried at 45°C for 8 hours followed by 65°C for 16 hours, or were freeze dried. For malting on the 50 kg scale, malting drums were used and conditions were adapted to reflect that of a commercial malting. In addition, this malting scale yielded sufficient material for pilot scale kilning. The kilning conditions were adapted to suit the investigation, and samples could be taken from various positions through the kiln bed.

Folate analysis.
Folates were analysed by the microbiological method, which is a standard method in the food industry. Samples were milled, then incubated in a buffer containing amylase and a hog kidney deconjugase in order to extract the folate from the malt material. A final incubation with a protease was also included, to make sure that the folate extraction was complete. This extract was then incubated with *Lactobacillus casei*, which requires folate for growth. The extent of bacterial growth

could therefore be related back to the folate content of the cereal by means of a standard growth curve.

Key Results

Survey of commercial malts and malted products

Several commercial maltings provided samples of a range of their products in order to benchmark their folate content. The main emphasis of this survey was on brewery related malts i.e. ale and lager, as well as the specialty malts such as crystal or high diastatic potential (HDP) malts. However, the survey also included food malts, malts from cereals such as oats as well as the maltings co-product roots.

The survey showed that ale and lager malts contained in the range 2-4 mg folate/kg, with average values for these malts being 2.8 mg/kg for both malt types. Similar figures were seen for other malted cereals such as oats, wheat and rye as well as for more processed products such as spray malts. However, the value for HDP malts was noticeably higher than for these other malted products, with an average folate content of 4 mg/kg (Figure 1).

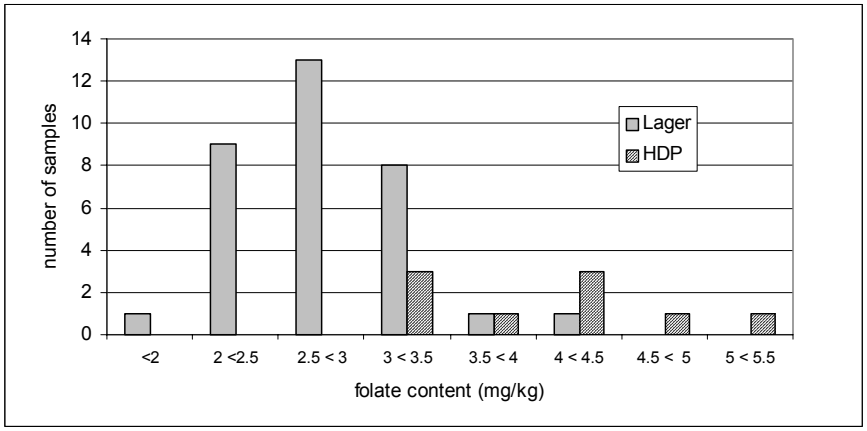

Figure 1: Comparison of the levels of folate in commercially produced lager and HDP malts

The higher levels in HDP malts are probably due their increased embryo development, and this will be discussed below in relation to the work with the influence of the malting process on folate content.

At the other end of the spectrum, some of the specialty malts were lower in folate content. For example, crystal malts contained about half the folate levels of ale and lager malts whereas in darker malts e.g. chocolate malts, folate was undetectable. From more detailed research on the production of these malts, the damaging effects of heating seemed to be responsible for these low levels.

Perhaps the most unexpected results from the survey work, were the high levels of folate which were found in roots. These folate levels sometimes reached up to 30 mg /kg, suggesting that this co-product is a very rich source of folate (Figure 2). Since folate synthesis takes place in the embryo, it is reasonable to explain this observation by suggesting that the root portion of the embryo should have also high concentrations of folate. However, of particular interest here is that the folate in roots survives the malting, kilning and the de-rooting processes to yield a high folate product. Therefore, we can conclude from this study that roots have a good nutritional value and it raises the possibility that this co-product might be developed into new functional food-type products.

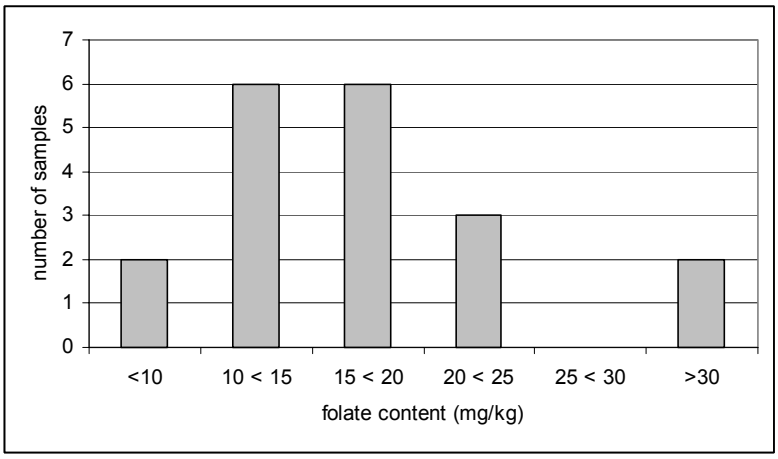

Figure 2: The distribution of folate content in a set of root samples

The error on folate measurements is ± 5% (see methods)

The malting process increases folate content.

The typical level of folate in unmalted cereals is between 0.5-1.0 mg/kg. This implies that the malting process itself increases the folate level in cereal 2-3 fold. By taking samples at each day of malting, it was possible to observe this process in more detail. Although there was some variation between varieties, as a general rule folate content increased rapidly for the first two days of germination, and

then continued to increase at a slower pace until kilning. It was also interesting that the kilning process itself did not appear to be unduly destructive to the folate content, and at most a 10-20% decrease was observed at this stage (Figure 3).

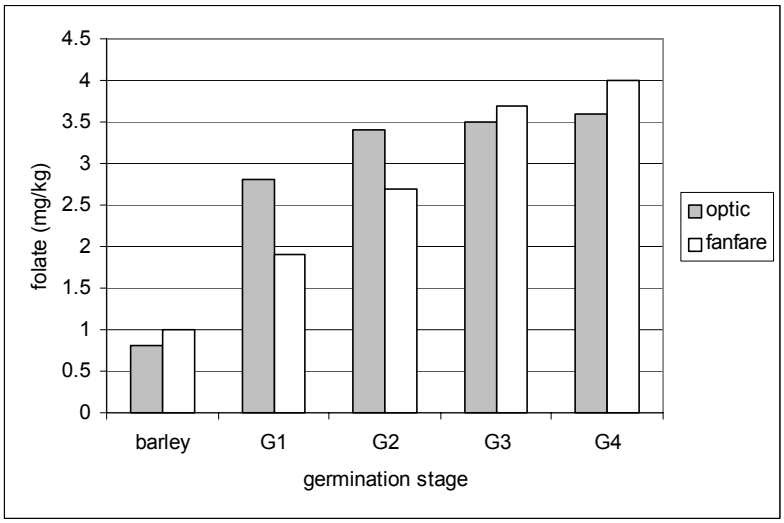

Figure 3: Patterns of folate accumulation in and ale and lager malt

The Fanfare barley was malted as an ale (with addition of GA), and the Optic was malted as a lager. Folate values are ± 5%

One of the aims of this project was to determine how malting conditions might affect the final folate content of the malt. Therefore, we tested the effects of both speeding up germination by the addition of gibberellic acid, and slowing down germination by the addition of bromate, The data showed that gibberellic acid caused a much more rapid and extensive accumulation of folate, whereas the presence of bromate was inhibitory. This suggested the level of folate in malt was connected to the extent of germination. This fitted nicely with the observation above that the HDP malts, which have a more extensive germination than ale and lager malts, also had a higher folate content.

The possibility that gibberellic acid treatment, or other methods to encourage embryo growth, could lead to the production of high folate malts was also investigated. In this case we found that the folate generated during this accelerated germination period was not stable to kilning, and so this increase in folate synthesis was not reflected in the final malt (Figure 4). This result may imply that folate made under these conditions is not stabilised by binding to proteins in the cell, and may therefore be more vulnerable to heat. Obviously this is an academic point, and was outside the scope of this

investigation. Extensive work with a range of kilning conditions was not able to demonstrate a better kilning strategy for folate preservation.

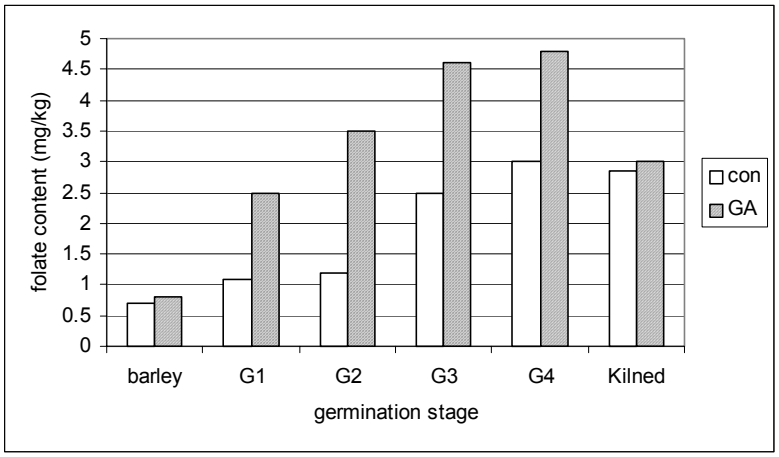

Figure 4: The effect of GA on the accumulation of folate during malting.
An Optic malt was the starting material. Folate values are ±5 %.

Varietal differences and the effect of barley growth conditions

A great range of folate contents was seen in malted products, but it was not clear whether these differences were due to variations in malting and kilning conditions alone. It was also possible that both the barley variety as well as its growth conditions might have an influence on folate content.

In our initial work, a set of barley varieties grown at the same site was examined. After malting these barleys under identical conditions, some barley varieties were found to have doubled their folate content whereas in others it was more than tripled. This suggested the some barley varieties might be able to increase their folate content more than others. However, in order to establish whether there is an effect of variety on folate content of the malted barley, a larger experimental set of barleys was required so that a statistical analysis could be performed. This set of barley contained 60 samples with the varieties Optic, Chariot, Cellar and Tavern.

After malting these samples, a statistical analysis of folate contents suggested that there was indeed a varietal effect on folate accumulation. For example, in this case Tavern produced significantly higher folate malt compared to Optic (Figure 5). Therefore, it could be concluded that the folate content of

malts is to some extent dictated by the variety. This result is not unexpected, since one would expect such traits to be under genetic control. However, it should be emphasised that although genetics may play a role in folate accumulation, malting conditions are also a very influential factor, and so it cannot be said that all Optic malts will have lower folate contents than all Tavern malts.

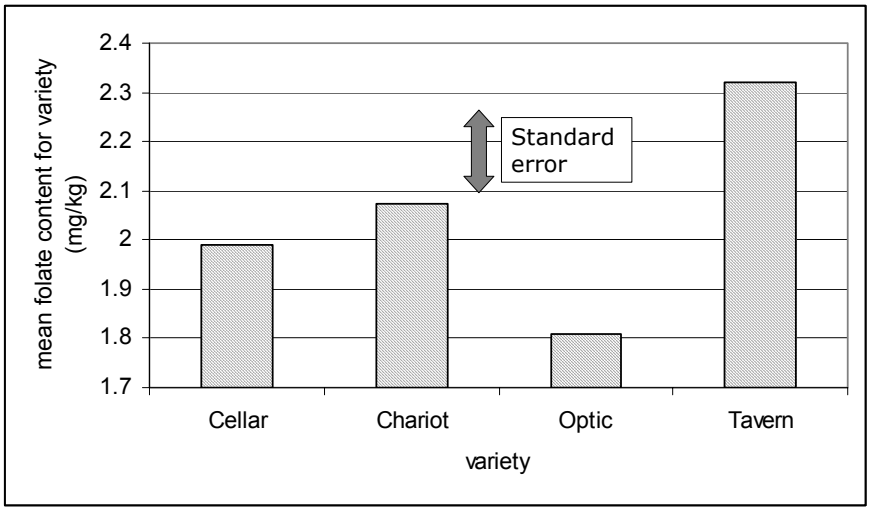

Figure 5: The mean folate content of Cellar, Optic, Chariot and Tavern malts.
The samples analysed were malts from barleys grown at a single site at a variety of seedrates. The data are shown with an indication of the standard error of the grand mean.

Another factor that was investigated in this project was the influence of growth conditions on folate accumulation during malting. Again, in order to allow for a statistical analysis, a set of 60 Optic samples was investigated, which contained samples grown at a single site but under various seed rates, Nitrogen and fungicide application. The data suggested that neither seedrate nor fungicide application had any influence on the folate content of the malt. However, an effect of nitrogen application was seen, with higher (150 kg N/ha) rates of nitrogen application favouring production of a higher folate malt (Figure 6). This result could indicate that seeds grown with higher rates of fertiliser are perhaps better able to store nutrients and proteins during seed formation, which then allows a faster rate of folate synthesis on germination.

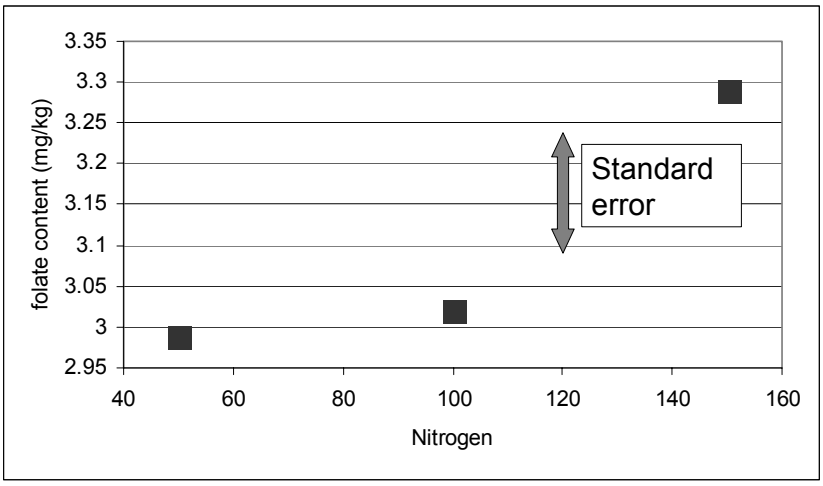

Figure 6: The relationship between nitrogen application and folate content in malt.
The malts were from Optic barley, grown at a single site at various N and fungicide applications and seedrate. The data for N application only are shown, together with an indication of a standard error of the grand mean.

Conclusions

The aim of this two-year project was to gain an overview into the folate content of malt and malting related products, and look at the factors which might influence them. From our survey work of malted products, it was clear that malts are generally a very good source of folate with levels two to three times higher than that seen in unmalted cereals. Different cereal types, food malts and brewing malts fell into the range of 2-4 mg folate/kg, although lower levels were seen in high colour products. Perhaps most intriguing were the high levels of folate in the roots, which are a co-product of the malting process. This suggested that roots might be considered for use as a nutritional supplement being a excellent natural source of folate.

The malting conditions themselves have an influence on the folate content of malted cereals. The extent of germination appears to be a key issue, and for products where a more extensive germination has been carried out there is also a higher level of folate. The HDP malts were consequently a good example of a high folate malt. On the other hand, whereas kilning conditions did not seem to be an influential factor on folate content, the heating processes used to produce darker colour malts were destructive to this vitamin.

Work with varietal sets suggested that barley variety also had an influence on the accumulation of folate during malting, and indicated that some genetic factors come into play during folate accumulation. In other words, a high folate content in the malt is in part an inherited characteristic. However, the folate content of malts can also be influenced by the growth conditions and increasing the levels of this vitamin by maintaining high levels of nitrogen application on the field could be possible.

In conclusion, the folate content of malts depends on several factors, including growth conditions, variety as well as malting conditions. In the future it should therefore be possible to produce high folate malts by optimisation of all of these factors.

Implications

The public is interested in foods that are naturally high in vitamins – the health-enhancing functional foods. The malting process lends itself to producing such products, by simply allowing the germination process to naturally enhance vitamin levels. In this short project we have been able to show that all of the currently commercially produced malts have naturally high levels of folate and could be marketed with the health-conscious market in mind.

Perhaps of most interest for maltsters is the discovery that roots are a very rich source of folate. This gives some scope to maltsters interested in developing new products, and can potentially increase the value of this co-product as it opens up a new market for them. It seems probable that roots will also be a rich source of other vitamins which may be potentially healthy ingredients, and it may be worth the maltsters investing more heavily in characterising the health potential of this co-product.

This project also has a lot of interest for the brewing industry. The folate in malt is stable during the brewing process and is carried through to the final beer. Since brewers are also interested in enhancing the healthiness of their beer, they will therefore be interested in using high folate products. Maltsters may therefore wish to emphasise the folate content of their products and monitor these as a selling point for the breweries. Custom made malts for producing high folate beers may also be a possibility. Work on malting conditions in this project suggests that this should be done through encouraging a more extensive germination. Obviously the brewer will need to make other adjustments of the grist and brewing conditions to allow for such a product.

Finally, there are some implications here for the farmers and barley breeders in terms of developing new varieties or improving growth conditions with a view to improving folate content. This project

suggests that genetic factors do come into play, with some varieties simply being better at synthesizing folate than others. The implication for the barley breeder is that this trait could be bred for, and it may be worthwhile to conduct a wider survey of the relative merits of different UK varieties as a guide to the best breeding lines. For the farmers, this project suggests that there is an additional payback for higher levels of nitrogen application in the field, which could perhaps be monitored and considered as 'added value' when marketing the seed.

In summary, in the current climate of health foods, the malting process has a lot to offer in the development of high value products. This project has made a start on identifying some of the areas that might be appropriate for further research and development.

The folate (vitamin B9) content of commercially available malted products and co-products

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ABSTRACT

When cereals are malted, the levels of folate (vitamin B9) increase. The extent of this increase is affected by the malting conditions as well as the barley variety. Hence the levels of folate in commercial products vary significantly. The aim of this study was to survey the folate content of commercially available malted products and co-products produced from the 2001 and 2002 harvest. The data showed that the folate contents of ale and lager malts were similar, containing an average of 2.8 mg folate / kg . The high diastatic potential malts had an even higher folate content, with an average value of 4.0 mg/kg. In contrast, darker roasted products and crystal malts had much lower folate values, with folate in the darkest of products being undetectable. Trials with roasting suggested that when the product reaches a temperature of about 200°C, the folate in the cereal starts to degrade. The survey also showed that the malting co-product roots, were a very rich source of folate, containing levels between about 10 and 30 mg folate /kg. This suggests that roots might be considered for the production of naturally vitamin-rich food products in the future.

INTRODUCTION

Cereals are a good source of vitamins in the diet, and contain especially high levels of B vitamins. For example, folate (vitamin B9) is one of the vitamins most likely to be lacking in Western diets and is present at high levels in cereals.

Early research (Finney, 1982) suggested that the level of many vitamins increased during germination, which would imply that malted cereals may be a better source of vitamins than unmalted cereals. By analysis of various malted products as a part of this HGCA investigation, it was possible to demonstrate that this was indeed the case, with malted cereals typically having 2-3 times the levels of folate as unmalted cereals. The purpose of this HGCA project has therefore been to establish how growth conditions, barley variety and malting conditions all affect the folate levels of the finished malt. The data have suggested that many of these parameters are influential, and a full description of this work is detailed elsewhere in this project report.

One of the key areas identified as a part of this project was to establish the folate content of a range of commercially available malted products and co-products, to provide those in the industry with more information of the nutritional properties of their products. This survey work is reported in the following paper.

MATERIALS AND METHODS

Survey Samples.

The following maltsters kindly provided commercial samples of malted products and co-products for the survey: Crisp Malting Group Ltd., Simpson's Malt, Muntons and Paul's Malt. Samples provided included both brewing and non-brewing malts, so that food malts and non barley malts were included in the survey.

Folate analysis

Folates were analysed by the microbiological method, which is a standard method in the food industry. Malt samples were milled, then incubated in a buffer containing amylase and a hog kidney deconjugase in order to extract the folate from the malt material. Roots were treated in the same way, except that they were ground with a mortar and pestle before analysis. A final incubation with a

protease was also included, to make sure that the folate extraction was complete. This extract was then incubated with *Lactobacillus casei*, which requires folate for growth. The extent of bacterial growth could therefore be related back to the folate content of the cereal by means of a standard growth curve. This 'triple enzyme' method of folate extraction is adapted from Pfeiffer et al (1997). Due to the complex nature of this multi-step analysis, the error on folate measurements for malt was ± 5%.

Roasting

For the roasting study, samples were taken at time intervals during the production of a chocolate malt (colour 1600 EBC) at Simpson's Malt. Colour and moisture were determined by IOB Methods of Analysis (1997) 3.4 and 3.2 respectively.

RESULTS AND DISCUSSION

Survey of malted products

One of the main aims of this project was to provide information to the cereal industry on the typical levels of folate in malted products and co-products with a view to highlighting their nutritional value. Therefore, commercially malted products were collected from four UK maltsters in order to produce a set of samples for a survey of folate content. The set included 33 lager malt, 27 ale malt and 9 high diastatic potential (HDP) malt samples. In addition, samples of spray dried malts, crystal and chocolate malts, malt flours, distilling, melanoidin, food, rye, oat and wheat malts were analysed for folate content.

The survey set of ale and lager malts included samples from a wide range of barley varieties, malted from barley grown at several different sites. Not surprisingly, the folate content in the malts also varied quite widely with levels usually falling between about 2 and 3.5 mg folate/kg (figure 1).

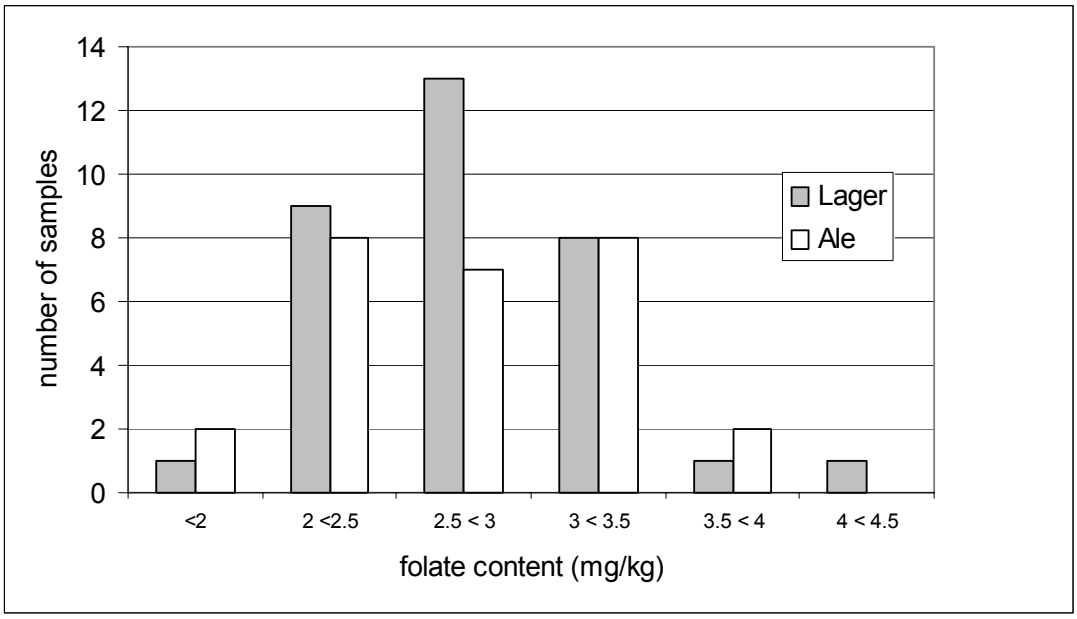

Figure 1: The distribution of folate contents in a survey of ale and lager malts.

For both ale and lager malts, the average folate content was 2.8 mg/kg, and a statistical analysis suggested that there was no significant difference between the folate content of these malts types (see below). However, when the ale and lager malts were compared to HDP malts, it was clear that the HDP malts had a higher folate content, ranging between 3 - 5.5 mg/kg (figure 2).

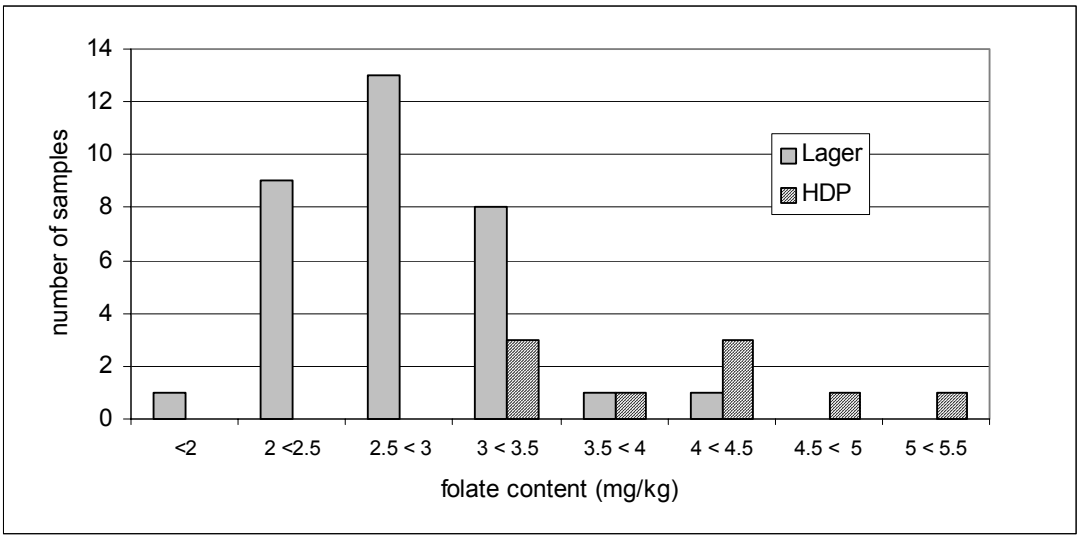

Figure 2: The folate content in a range of lager and HDP malts

Although the number of HDP malt samples was lower than ale and lager malts, reflecting their relative level of production, the numbers of all these samples were sufficient for a statistical analysis. This analysis is summarised in table 1 and figure 3, and shows that that HDP malts had a significantly higher folate content than both ale and lager malts (figure 3), but that ale and lager malts were not significantly different (table 1).

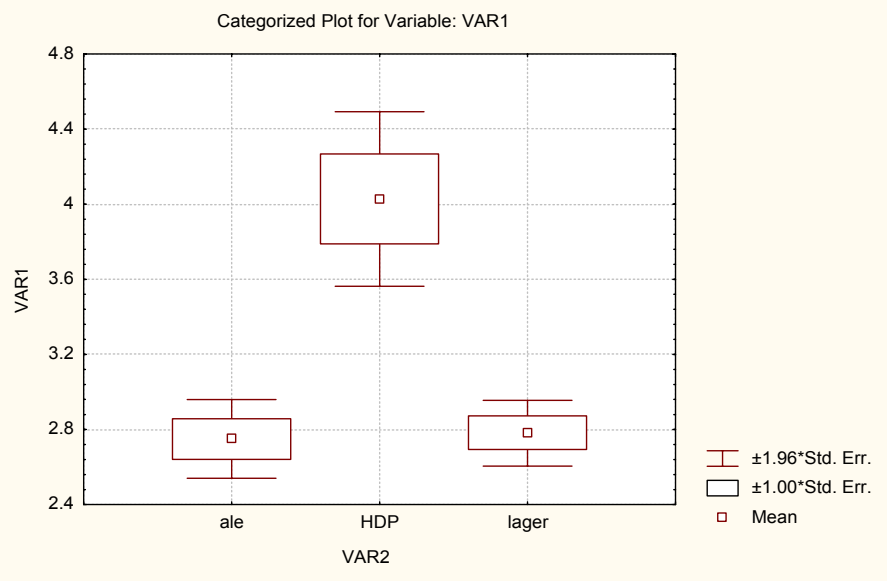

Figure 3: Average folate contents of ale, lager and HDP malts.

The figure shows the folate content as VAR1 (units mg/kg), the mean value for each malt type, and the variation about the mean. The higher variation in the HDP malts reflects the smaller sample size.

	Ale	HDP	Lager
Ale		0.00000*	0.834781*
HDP	0.00000*		0.00000*
Lager	0.834781	0.00000*	

Table 1. Least Significant Difference (LSD) test on the average folate values of ale, lager and HDP malts.

The data shown are probability levels for samples being different, with numbers close to zero indicating a high probability that samples are different. In the table, an asterisk denotes samples that are statistically different from each other.

These results match well with those from our pilot studies on the effects of malting conditions on folate content, in which the folate levels in lager and ale malts were found to be very similar. On the other hand, it was also shown that folate levels were higher when there was a greater amount of embryo development. Therefore malts such as HDP malts, where a higher level of modification is required, would be expected to have correspondingly higher levels of folate.

As mentioned above, the survey included many other types and styles of malts. None of these were analysed in any great numbers, but it was found that in all cases folate levels lay between 2 and 3.5 mg folate/kg. It was therefore considered unlikely that the folate levels in any one of these malt types were different from barley malts, although higher sample numbers would be needed in order to confirm this. With the limited numbers of some of these malts available, it was not a practical task to undertake in this investigation.

To make the survey as wide as possible, more processed malted products were included to see if further processing might have a detrimental effect on the levels of this vitamin. The results from spray malts and flours are shown in table 2.

Sample	Folate content (mg/kg)
Spray: 'light'	2.6
Spray: 'amber'	2
Spray: 'dark'	2.5
Spray 'extra dark'	2.2
Barley flour	2.7
HDP flour	3.5
Wheat Flour	2.2

Table 2. Levels of folate in selected malted products

The error measurement on folate content is ± 5% (see methods)

The spray malts might be expected to have a lower folate content than the cereals, since they have been subjected to an extraction and drying process. Similarly, the flours have been milled and exposed to oxidising conditions that might also be damaging to folate content. Given that the folate levels of these more processed products were still reasonably high, it can be concluded that this further processing is not excessively destructive to folate. On the other hand, the data would suggest that some folate has been lost during processing since these products would be used in lesser quantities than malt to achieve the same level of extract.

To summarise, of the products included in this survey, the HDP malts showed the highest folate levels and have therefore good potential to be marketed as high nutritional products. However, the survey also included the co-product roots, and these had a much higher folate level than all the malt samples in the survey.

Roots are an excellent source of folate

Several malt root samples were analysed for folate content and were in all cases found to contain higher levels of this vitamin. From the 17 samples analysed, folate levels varied between about 10 and 30 mg/kg which was approximately 10 fold higher than the levels seen in other malts samples (figure 4).

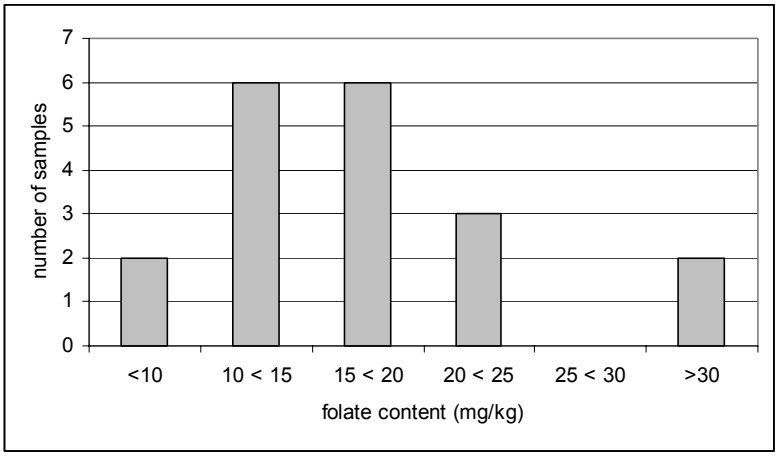

Figure 4: The distribution of folate content in a set of root samples.
The error on folate measurements is ± 5% (see methods)

The high levels of folate in roots were expected, based on the distribution of this vitamin within the seed. As discussed above, folate synthesis is carried out in the embryo itself. Therefore roots, which are essentially embryo material, might be expected to have a high vitamin content. For standard malts, the seed is milled before analysis which effectively 'dilutes' the embryo material with the starchy endosperm fraction, although extensive seed dissection and analysis was not carried out to confirm this. However, we can conclude from this study that roots have a good nutritional potential. This

raises the possibility that this co-product might be developed into new products, to take advantage of this natural source of vitamins.

Folate levels in high colour products

Several high colour products were included as a part of the survey. In general these contained lower levels of folate than the malts, and in the darkest samples folate was not detectable (Table 2).

Sample	Colour (°EBC)	Folate (mg/kg)
Malt	4	3.3
Low colour crystal	6	2.4
High colour crystal	176	1.8
Low coulour roasted barley	360	Not detectable
Chocoalte malt	1000	Not detectable

Table 2. Folate levels in coloured malts

The error range on the folate determination is ± 5% (see methods).

These results suggested that the higher temperatures needed to produce these crystal malts and dark roasted products were destroying the folate. In order to investigate this further, samples were taken at intervals during the production of a chocolate malt and analysed for folate. The product's temperature, colour and moisture were also recorded to establish the conditions at which the folate became unstable. The results suggested that the folate started to degrade at about 30 min into the roasting process, at which point the colour was still close to zero (Figure 5). Indeed, the folate was almost completely degraded by the point at which the colour formation reactions started in the malt.

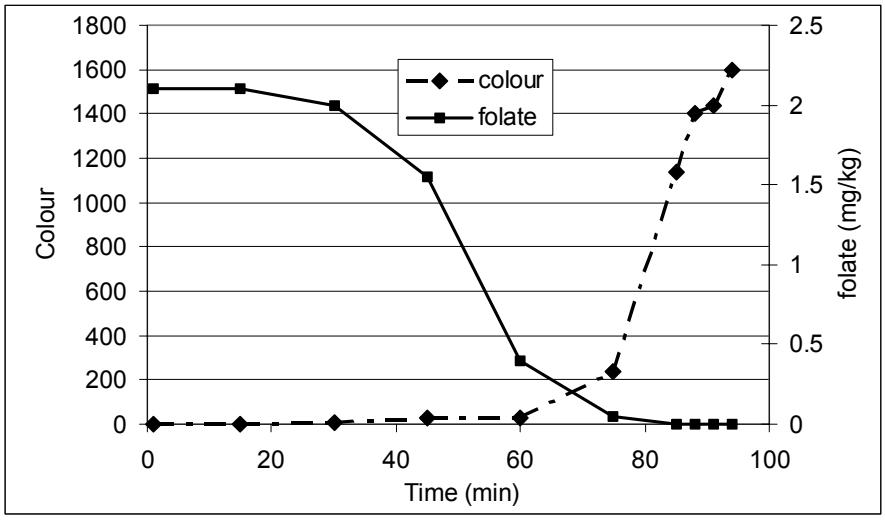

Figure 5. Comparison of colour formation and folate levels during the production of a chocolate malt

By measuring the temperature of the product, it could be seen that folate loss started at a temperature of about 200°C (Figure 6). These results indicate that under roasting conditions, folate destruction precedes colour formation, suggesting that it is not possible to produce a high- colour roasted malted product which retains its vitamin content.

On the other hand, the conditions for the production for colour in crystal malts are more gentle and are not as destructive to folate content. For example, from the data in Table 2, we can see that a crystal product with a colour of 176 was produced with only a 30% loss of folate. The reason for this difference between roasted and crystal malts may lie in the process itself. In the production of crystal malt a green malt is stewed, which effectively increases the levels of Maillaird reaction precursors (amino acids and sugars) in the grain. By increasing the levels of amino acids and sugars in the malt, colour reactions can take place at lower temperatures, which in this case results in the preservation of the vitamin content. Therefore, not all coloured products are completely lacking in folate, and for non brewing purposes maltsters may consider utilising crystal malts as a darker-coloured nutritious product.

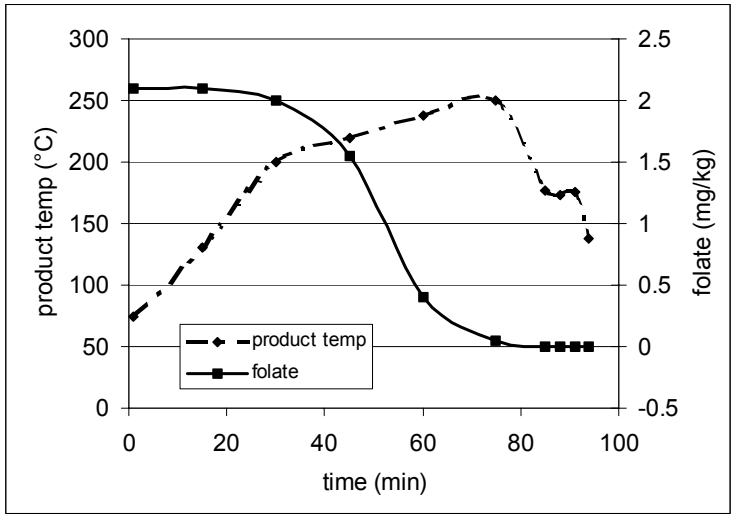

Figure 6: The relationship between folate levels and product temperature during the production of chocolate malt.

CONCLUSIONS

The aim of this part of this HGCA-funded project was to establish the folate content of UK-produced malted products, with a view to providing a guideline to their nutritional value. The results of this survey showed that levels of 2.8 mg folate/kg were typical for brewing malts, and other malted cereal products. These levels are 2-3 fold higher than those found in unmalted cereals, suggesting that the nutritional value of malt should not be overlooked. Perhaps the most significant result from this work was the discovery that HDP malts were especially rich in this vitamin and could perhaps be marketed with a view to emphasising their nutritional value. In addition, the very high levels of folate in the Maltings co-product, roots, offers potential for the development of new nutritional-based products for the marketplace.

ACKNOWLEDGEMENTS

The authors would like to thank all the Maltsters who contributed samples for the survey, or gave us access to their facilities. Catherine O'Shaughnessy is thanked for her help with the roasting work, Karin Pawlowsky for performing the statistical analysis and also Chris Booer for his ever useful insights and suggestions during the project. This project was funded by the HGCA (no. 2366), who are also thanked for their financial support.

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Pfeiffer, C.M., Rogers, L.M. and Gregory, J. F. (1997) J. Agric. Food Chem **45**: 407-413

Increases in the folate (vitamin B9) content of barley during germination

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ABSTRACT

Cereals are a good source of folate (vitamin B9), a vitamin which is often lacking in Western diets. However, malted cereals are an even better source of folate, since the germination process itself raises the folate content of cereals. The aim of this project was to monitor the folate increase during germination, and look at how conditions in the Maltings affect this process. When barley or wheat was malted on the pilot scale, a 4-5 fold increase in folate content was observed. By monitoring folate content on a daily basis it could be seen that the folate increase was faster under certain conditions. The addition of gibberellic acid during malting increased the accumulation of folate, whereas the application of bromate inhibited this accumulation, suggesting that folate accumulation was linked to embryo development. Pilot kilning studies showed that the folate was stable to typical kilning temperatures. The exception to this was after application of gibberellic acid, where kilning seemed to be destructive to folate. The data suggested that the folate synthesised under accelerated conditions, such as after application of gibberellic acid, is not as stable as that synthesised under standard germination conditions. However, malts high in folate content might be achieved by combining an increase in embryo development with gentler kilning conditions.

INTRODUCTION

Cereals are a good source of vitamins in the diet, and contain especially high levels of B vitamins. For example, folate (vitamin B9) is one of the vitamins most likely to be lacking in Western diets and is present at high levels in cereals.

Early research (Finney, 1982) suggested that the level of many vitamins increased during germination, which would imply that malted cereals may be a better source of vitamins than unmalted cereals. By analysis of various malted products as a part of this HGCA investigation, it was possible to demonstrate that this was indeed the case, with malted cereals typically having four times the levels of folate as unmalted cereals. The purpose of this HGCA project, has therefore been to establish how growth conditions, barley variety and malting conditions all affect the folate levels of the finished malt. The data have suggested that many of these parameters are influential, and a full description of this work is detailed elsewhere in this project report.

One of the key areas identified as a part of this project was to establish how the increase in folate content is affected by the malting conditions. This question was approached by varying the malting conditions in the small (300g) and pilot (50 kg) scale, and measuring the folate content of the products. The data described here suggest that folate levels are linked to the extent of embryo development.

MATERIALS AND METHODS

Malting conditions

For barley samples malted at the 300g scale. Samples were steeped for 8 hours, given a 16 hour air rest, then steeped again for 24 hours. The steeping liquor was then removed and germination was for 4 days at 16°C. After malting samples were either oven dried at 45°C for 8 hours followed by 65°C for 16 hours, or were freeze dried (as indicated in the text). Application of gibberellic acid or bromate were as indicated in the text.

For barley samples malted on the pilot 50 kg scale, germination conditions were set to provide a close approximation to those in commercial plants and were adjusted according to the barley variety being

malted. Samples (100g) were taken at daily intervals and either freeze dried or oven dried as described above. On the final day of malting, the remaining material was kilned in the pilot plant, and where necessary, samples were taken at the top, bottom and middle of the kiln bed.

Folate analysis

Folates were analysed by the microbiological method, which is a standard method in the food industry. Samples were milled, then incubated in a buffer containing amylase and a hog kidney deconjugase in order to extract the folate from the malt material. A final incubation with a protease was also included, to make sure that the folate extraction was complete. This extract was then incubated with *Lactobacillus casei*, which requires folate for growth. The extent of bacterial growth could therefore be related back to the folate content of the cereal by means of a standard growth curve. This 'triple enzyme' method of folate extraction is adapted from Pfeiffer et al (1997). Due to the complex, multistage nature of this extraction, all folate values are quoted as ± 5%.

RESULTS AND DISCUSSION

The typical levels of folate in unmalted cereals are 0.5-1 mg/kg, whereas the typical levels in malted cereals are 2-3.5 mg folate/kg. This suggests that at some point in the malting process there is a substantial synthesis of folate. In order to establish at what point this synthesis of folate occurs, a sample of Optic barley was malted on the pilot scale, and folate levels were monitored on a daily basis. In order to eliminate any possible influence of oven drying, the samples collected were freeze dried to minimise any loss of folate. Under these conditions, the folate content of the barley started to increase on the first day of germination, and continued to increase for the full four days of the malting period (figure 1).

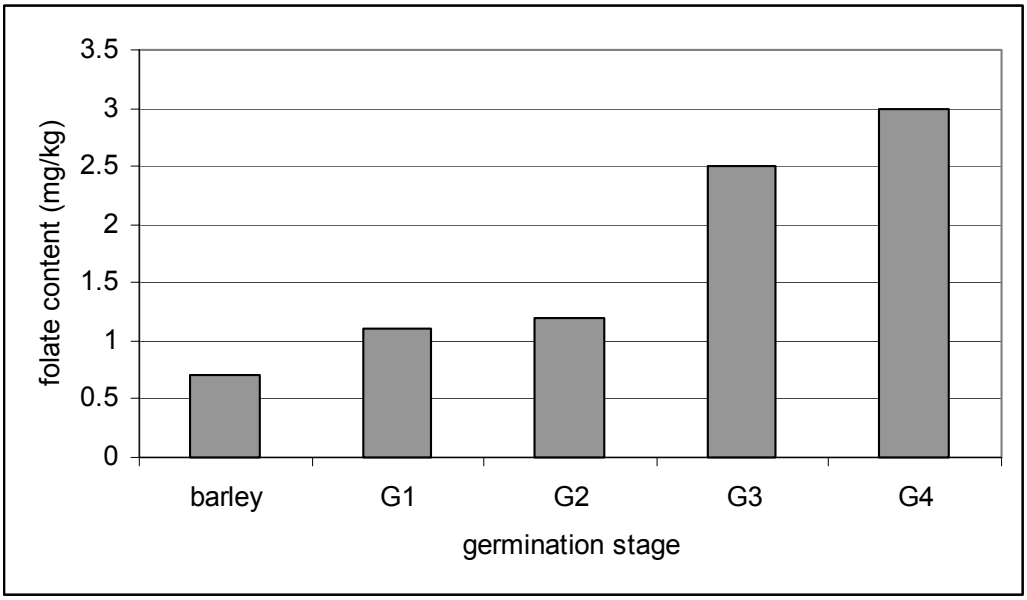

Figure 1: The increase in folate content during the germination of barley

In this experiment, Optic barley was malted. The folate values are ± 5% (see methods)

Overall, the increase in folate content during malting was approximately four fold, which is a substantial rise and emphasises the superior nutritional value of malted over unmalted cereals.

This increase in vitamin synthesis on germination is not confined to barley, and is likely to be similar for all cereals. For example, we also carried out a similar study with a wheat malt, since this is also utilised by the brewing industry for wheat beer production. Figure 2 shows that the increase in folate content during the malting of wheat was also substantial over the four day period. Although the overall increase was slightly higher in wheat compared to barley, we cannot make any generalisation about the relative folate accumulation in these two types of cereals since we have not tested enough barley and wheat samples. Data presented elsewhere in this report suggest that both variety and growth site have an influence on the extent to which folate is accumulated during germination, and it would therefore require a much more extensive study to establish if there are any significant inter-cereal differences.

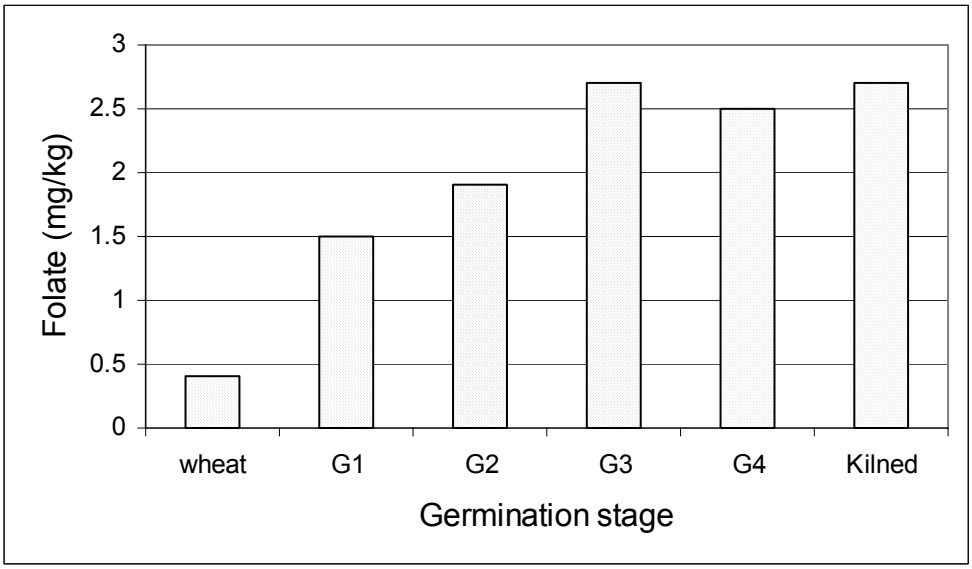

Figure 2: The increase in folate content during the germination of wheat

Folate values are ± 5%.

Considering barley alone, the conditions for malting vary considerably according to the type of malt to be produced e.g. ale vs lager. In order to make a side-by-side comparison of how typical ale and lager malts might perform with respect to folate accumulation, folate was monitored in the production of a Fanfare ale malt and an Optic lager malt. Other than the differences between variety and malting conditions in this trial, the Fanfare malt was also treated with gibberellic acid (GA) which is a common treatment applied to make ale malts in the UK. Figure 3 shows that both malts accumulated folate over the course of four days, and that both increased their folate content by approximately four fold. However, when compared on a day-by-day basis, the accumulation of folate was slightly more rapid earlier during the malting process in the lager malt compared to the ale malt. Again, given that these experiments require the pilot scale, it was not practical to extend this work to see to what extent these differences were a consequence of barley variety. On the other hand, it was possible to design an experiment to specifically test if the presence of GA has an effect on folate accumulation during malting.

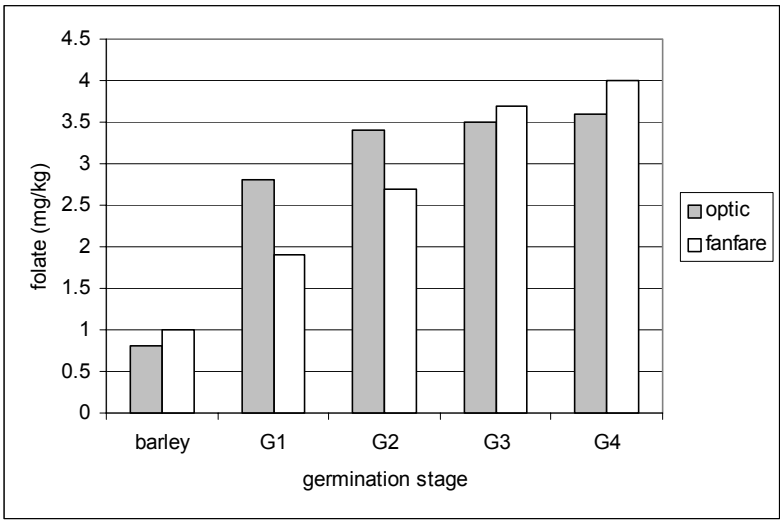

Figure 3: Patterns of folate accumulation in and ale and lager malt

The Fanfare barley was malted as an ale (with addition of GA), and the Optic was malted as a lager. Folate values are ± 5%

To determine whether GA had an effect on folate accumulation, two pilot scale maltings were run side-by-side under identical conditions with the variety Optic . For one of these samples, GA was applied during germination. Every day during malting, samples were taken for folate analysis as described above, and samples were taken again after kilning. In this case there was a very clear difference in folate accumulation between the two malting conditions (figure 4). In the presence of GA, folate accumulation was much more rapid and more extensive during germination. In fact the untreated sample had a folate content of only 3 mg/kg at the end of germination compared to the GA-treated sample where folate reached a level of 4.7 mg/kg. Since all other conditions were identical during malting, the data indicated that GA had a direct and stimulatory effect on folate accumulation.

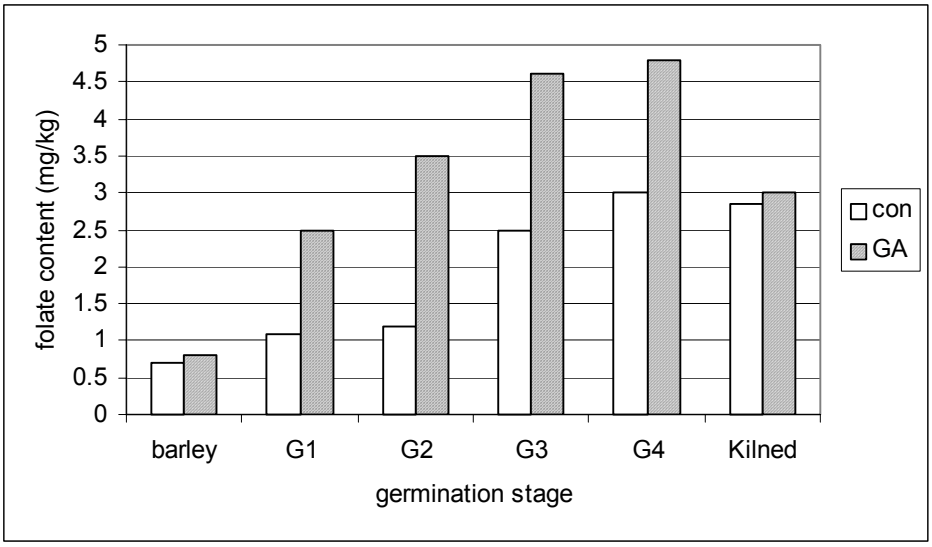

Figure 4: The effect of GA on the accumulation of folate during malting.

Optic barley was the starting material. Folate values are ±5 %.

Another clear difference between the treated and untreated samples was seen at the stage of kilning (figure 4). Whereas in the control sample kilning made no significant difference to folate content, in the sample treated with GA kilning caused a 40% loss of folate. This rather drastic effect of kilning on folate content after GA application was unexpected, but may be due to some unusual consequences of GA application on folate stability. Folates are usually found in the cell bound to protein, and these proteins may confer some degree of protection to heat. Once folates are extracted from proteins they tend to become less stable and are therefore more readily degraded. It can be speculated that when GA causes much larger quantities of folate to be made, then the proteins required for stabilising this vitamin in the cell are in too short supply to bind all the folate directly. This would leave a proportion of folate 'unbound' in the cell and susceptible to breakdown during kilning. Another consideration is that folates have a polyglutamate (amino acid) side chain which also increases the stability of this vitamin. Again, it is possible that either the enzyme responsible for adding this side chain to the vitamin, or the supply of glutamate, may become too limiting to function normally under the conditions of GA application. The result of such a limitation could possibly cause a proportion of the folate to be relatively unstable when exposed to kilning temperatures. The mechanism by which GA application generates a pool of 'unstable' folate is very interesting and would make an excellent topic

for further academic research. But from an applied point of view, such an investigation was considered too detailed for this project, although it clearly has consequences for developing potential strategies for increasing the folate contents of malt.

It should be emphasised that we observed no significant loss of folate in the control sample of this experiment (figure 4), and so normal kilning conditions do not seem to pose any problems as far as folate content is concerned. This result can also be seen for the malting of wheat in figure 2. As a part of this project, we performed a range of kilning trials to test for possible gradient effects in folate content across the kiln bed, and to test for optimal conditions. However, the effect of kilning on folate content was so minimal on ale and lager malts, that it was concluded that there was no benefit to optimising kilning conditions for standard malts. While unexciting, this result is reassuring in that most of the folate made during malting is likely to be almost completely preserved in the finished malted product, with the exception of malts treated with GA. However, the GA-treated samples still had a high folate level, which was in the same range as untreated malts. The practice of GA application could not therefore be considered to be detrimental to folate content in the context of commercially available products.

The stimulation of folate production with GA accumulation seen in figure 4 also suggested that folate accumulation is linked to embryo development, with stimulated embryo development leading to increased folate production. In order to test this hypothesis, the effect of the additive bromate on folate accumulation was tested. Bromate has the ability to inhibit protein synthesis during germination, and would therefore be expected to slow down many of the biochemical pathways that are being activated as the seed grows. Therefore a small scale (300g) experiment was carried out where barley was malted at several different concentrations of bromate (figure 5).

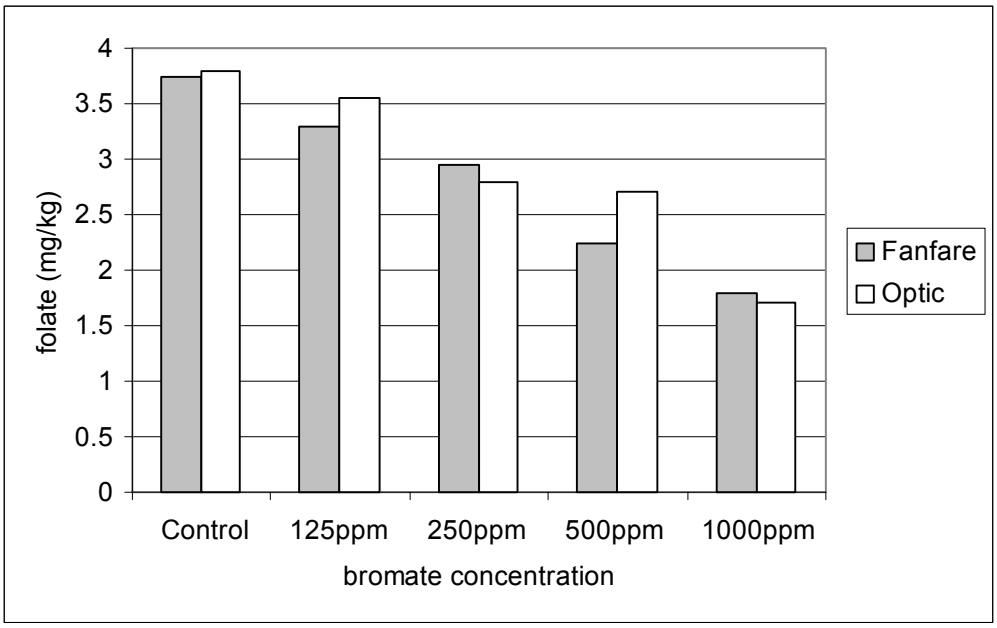

Figure 5: The effect of bromate addition during malting on the folate levels in Fanfare and Optic malts.

Malting was on the 330g scale. Folate values are ± 5%

The data showed that in the absence of bromate, the folate levels in Fanfare and Optic malts were similar (approximately 3.7 mg/kg) after malting. In the presence of bromate, the folate levels in both malts were reduced in a dose-dependent manner, with bromate at 1000 ppm being the most inhibitory. This result therefore suggests that protein synthesis, and presumably embryo development, is a key factor for determining the accumulation of folate during germination.

This observation from experimental work fits well with the results from our survey of malted products in which we found that high diastatic potential (HDP) malts had a significantly higher folate content than ale and lager malts. Since HDP malts are usually germinated under more intensive conditions than either ale or lager malts it would be expected for them to have more embryo development and consequently a higher folate content. In addition, the much cooler kilning temperatures for HDP malts (usually in the region of 60°C as a maximum temperature) would help to preserve folate stability after malting. Our survey work showed that roots were also an excellent source of folate, with levels 10 times higher than those in malted cereals. This result is quite consistent with folate synthesis taking

place in the embryo, since roots are themselves a part of the embryo and are essentially free from the folate-poor endosperm and husk of the seed.

CONCLUSIONS

This pilot and small scale malting study has revealed some key factors which may affect the accumulation of folate during malting. First, folate accumulation is linked to the extent of embryo development, and second, if embryo development is 'pushed' too quickly, the folate produced may be less stable to kilning. Taken together, this work can provide some guidelines for maltsters interested in developing high folate malts as foods of high nutritive value. For example, a high folate malt could potentially be generated by the use of GA during malting followed by a much lower temperature and gentler kilning regime. Another factor that might be considered is the starting material. Work on variety and growth conditions reported elsewhere in this project suggest that some varieties are better at accumulating folate during malting than others, and that higher levels of nitrogen application could also be beneficial.

To summarise, the accumulation of folate during malting is a highly complex process and is affected by a wide range of factors. However, there are clear strategies that might be used if a maltster wishes to enhance the nutritional value of malted products.

ACKNOWLEDGEMENTS

The authors would like Jim Grant for technical assistance. This project was funded by the HGCA (no. 2366), who are also thanked for their financial support.

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Pfeiffer, C.M., Rogers, L.M. and Gregory, J. F. (1997) J. Agric. Food Chem **45**: 407-413

The effect of variety and growth conditions on the folate content of malted cereals

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ABSTRACT

When cereals are malted, the levels of folate (vitamin B9) increase. Although the extent of this increase is affected by the malting conditions themselves, in this study, we asked whether variety and growth conditions could also have an influence. Several sample sets of barley were used in order to address this question. The folate levels in malts prepared from a set of 9 varieties grown at a single site varied between 3.5 and 4.8 mg folate/kg. This suggested that some varieties may have more potential to accumulate folate than others. In order to analyse this hypothesis in more depth, the folate levels in malts prepared from a statistically designed sample set of 60 barleys (varieties Tavern, Cellar, Optic and Chariot) were measured. The data showed that there was indeed a statistically significant varietal effect, with some varieties accumulating more folate than others. This result suggested that there must be a genetic component to the ability to accumulate folate. In order to establish the effect of growth conditions, a statistically designed sample set of 60 (Optic) barley samples were also malted and folate levels were measured. These barleys varied in seedrate, nitrogen application and fungicide application. The data suggested that of these parameters, only nitrogen application had a significant effect on folate accumulation, with higher levels of application favouring higher folate levels in the finished malt. These results provide some strategies for the barley breeder and farmer as to how the folate content of malts might be naturally enhanced.

INTRODUCTION

Cereals are a good source of vitamins in the diet, and contain especially high levels of B vitamins. For example, folate (vitamin B9), is one of the vitamins most likely to be lacking in Western diets and is present at high levels in cereals.

Early research (Finney, 1982) suggested that the level of many vitamins increased during germination, which would imply that malted cereals may be a better source of vitamins than unmalted cereals. By analysis of various malted products as a part of this HGCA investigation, it was possible to demonstrate that this was indeed the case, with malted cereals typically having 2-3 times the levels of folate as unmalted cereals (detailed in this report). It was therefore of interest to establish which parameters affected the ability of the barley to accumulate folate, and look at the possibility of naturally producing high folate malts. Work on malting conditions revealed that embryo development and heat treatments were key factors affecting the extent of folate accumulation, and this work is also detailed in this report. However, the purpose of this part of the investigation was to look at the question of whether growth conditions and/or barley variety were also influential factors on folate accumulation.

MATERIALS AND METHODS

Barley

Several sets of barley were collected for this project.
The first sample set came from a single site (Rothwell) and consisted of both brewing and non brewing barley varieties, and were from the 2000 harvest. The varieties were as follows: Jewel, Regina, Pearl, Fanfare, Heligan, Artist, Static, Halcyon and Delibes.

The second and third sample set came from ADAS, and were grown at the BG Bridgets site under very closely defined conditions (also year 2000). Each sample set consisted of 60 barley samples, and these sets were designed in order to give sufficient statistical power to determine the effects on variety and growth conditions.

In the first of these sets, the barleys tested were Optic, Chariot, Cellar and Tavern. These were grown at the seedrates of 50, 100, 2000, 4000 and 8000 / m^2; nitrogen application was 100kg N/ha and Amistar Pro Unix was applied as fungicide for all of these samples.

For the second set to determine the effect of growth conditions, the following parameters were varied according to Table 1 below:

	Seedrate
1	100 seed/m^2
2	400 seed/m^2
	Nitrogen
1	50 kg N/ha
2	100 kg N/ha
3	150 kg N/ha
	Fungicide
1	Amistar Pro 2l/ha plus
	Unix 0.67 kg/ha GS 30-31
2	Opus 1.0l/ha plus
	Corbel 0.5l/ha GS 30-31
3	Amistar Pro 2l/ha plus
	Unix 0.67 kg/ha GS 30-31 +
	Amistar Pro 2l/ha GS 45-59

Table 1: Variations in seedrate, Nitrogen application and Fungicide application utilised to determine the effect of growth conditions on folate accumulation in germinating barley

Malting conditions

All barley samples were malted under identical conditions at the 300g scale. Samples were steeped for 8 hours, given a 16 hour air rest, then steeped again for 24 hours. The steeping liquor was then removed and germination was for 4 days at 16°C. After malting samples were either oven dried at 45°C for 8 hours followed by 65°C for 16 hours, or were freeze dried (as indicated in the text).

Folate analysis

Folates were analysed by the microbiological method, which is a standard method in the food industry. Samples were milled, then incubated in a buffer containing amylase and a hog kidney deconjugase in order to extract the folate from the malt material. A final incubation with a protease was also included, to make sure that the folate extraction was complete. This extract was then incubated with *Lactobacillus casei*, which requires folate for growth. The extent of bacterial growth could therefore be related back to the folate content of the cereal by means of a standard growth curve. This 'triple enzyme' method of folate extraction is adapted from Pfeiffer et al (1997)

Statistical Analysis

Statistical analysis was by the Genstat 3.5 programme (Windows)

RESULTS AND DISCUSSION

Experimental work had demonstrated that malting conditions influenced the rate and extent of folate accumulation during germination. The aim of this part of the project was to establish whether variety and growth conditions could also have an influence. The first sample set used to address this question came from the Rothwell site. This collection of barleys included both malting varieties (Pearl, Regina, Fanfare and Halcyon) as well as feed grade varieties such as Heligan. As such the samples included a wide spectrum of barley types which in principle would have been expected to give us the greatest variation in terms of folate accumulation during malting. Clearly, if we were not able to measure differences between such a diverse range of barley types, we could assume that varietal effects were not a significant factor in folate accumulation.

The first parameter measured was folate content in the unmalted barley samples, to determine if the endogenous levels of folates were different. The data showed that the folate levels were indeed different between the varieties, varying between 0.9 and 1.3 mg folate/kg (Figure 1).
However, it should be noted that with an error of ± 5%, these varietal differences were not substantial.

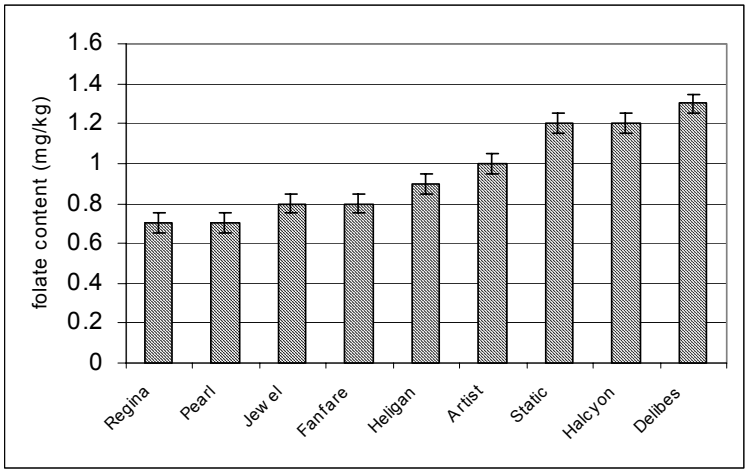

Figure 1. The folate levels in several barley varieties, grown at Rothwell

After micromalting (see methods) the samples were freeze-dried, in order to provide the gentlest and most non-destructive drying regime to the folate as possible. As expected, the folate levels were much higher in the malted samples, varying between 3.5 and 4.6 mg folate kg (Figure 2).

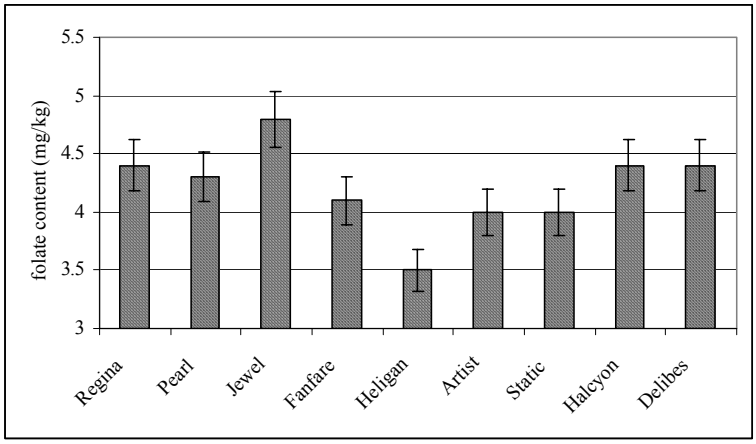

Figure 2. The folate levels in several barley malts. The barley samples were all grown at Rothwell, and micromalted under identical conditions

In order to gain a better comparison between the barley varieties, the increase in folate content during malting relative to that in the barley was estimated for each variety. This data is summarised in Table 2.

Variety	Folate increase relative to barley
Pearl	4.8
Regina	4.4
Fanfare	4.1
Heligan	3.5
Artist	4
Jewel	4.4
Halcyon	3.6
Delibes	3.6
Static	3.1

Table 2: The extent of folate increase on malting for a selection of varieties grown at the Rothwell site. Folate levels were measured both in the barley and corresponding malts. The folate increase was calculated as a factor comparing the malt to barley, with a value of 3 indicating that the folate levels tripled on malting.

It was concluded from this initial experiment that these barley varieties showed clear differences in their ability to accumulate folate when malted under identical conditions. Some varieties only tripled their folate content, whereas other were able to produce an almost five fold-increase. This suggested that further work to look more closely at varietal effects would be justified.

Although the above data suggest that varietal effects may indeed be important in determining the extent to which folate is produced during germination, this result cannot be considered as statistically valid, due to the limited amount of samples analysed. Therefore it was decided to use a sample set which had been specifically designed to give sufficient data to allow for a statistical analysis on this question. The sample set consisted of four barley varieties (Cellar, Optic, Tavern and Chariot) which were grown at a single site but at a variety of seedrates (see Materials and Methods). All of these barleys were malted under identical conditions, and then gently oven dried.

The folate contents of the malted barleys were statistically analysed for both an influence of seedrate as well as for an influence of variety. The analysis showed that seedrate had no significant effect on the malt folate content, whereas barley variety did appear to be an influential factor. Figure 3 shows the mean value for the folate content of each variety, and indicates the standard error of this measurement. The data suggest that the folate levels in Tavern malts were significantly higher than those in Optic, Tavern and Chariot malts. Whereas there was no significant difference between Cellar

and Chariot malts, both of these varieties' malts had a significantly higher folate content than the Optic malts.

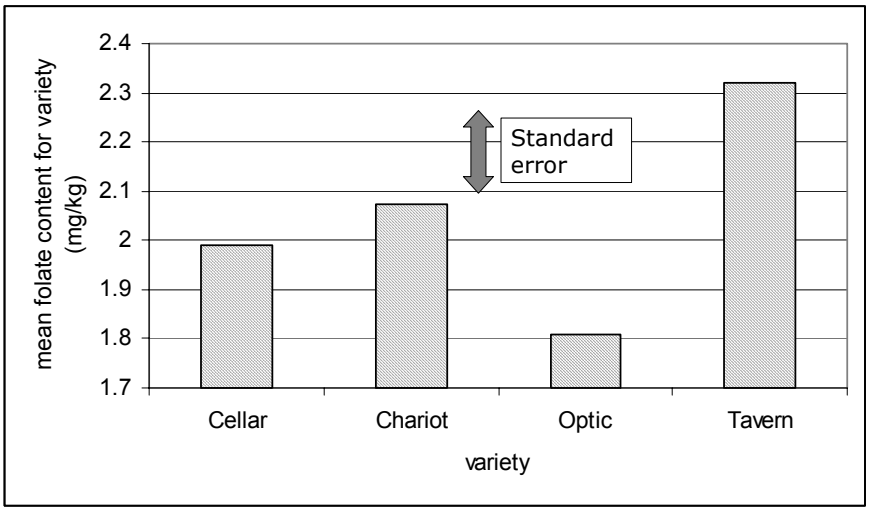

Figure 3. The mean folate content of Cellar, Optic, Chariot and Tavern malts. The samples analysed were malts from barleys grown at a single site at a variety of seedrates. The data are shown with an indication of the standard error of the grand mean.

These barley samples were also analysed for several other parameters as a part of the HGCA-funded project on Protocols to Control Malt Quality (no. 2294). The folate values in these malts were therefore also checked for correlations with the following parameters: Light Transflectance (LTm), germination capacity, germinative energy, water sensitivity, sieve distribution, growth delay and growth rate. However, there was no correlation between malt folate content and any of these parameters, suggesting that only variety had any significant on folate accumulation during malting.

The discovery of a varietal influence on folate accumulation indicates that genetic factors are important in determining the rate of folate accumulation in a germinating seed. This result is not entirely unexpected since vitamin synthesis requires the combined action of many different biosynthetic enzymes. Plant varieties might be expected to differ in the levels of these enzymes which are present in the seed, and in the speed in which the production of these enzymes are 'switched on' during germination. The response of a seed to external factors (water and sunlight) is also extremely complex, and would again be expected to vary according to the genetic background of the plant.

Although these results indicate that plant breeders may in the future be able to select for a trait such as 'rapid vitamin synthesis' it should be remembered that other factors affect the rate of folate accumulation e.g. malting conditions. Therefore, producing a high folate malt is not just a matter of selecting the variety, but also of optimising malting and kilning conditions.

As mentioned above, no correlation was seen between folate accumulation and seed rate, suggesting that this growth condition was not of importance to this study. However, clearly there are other growth conditions which might also have an influence on the folate accumulation in seeds during malting. Therefore, a second statistically designed sample set was analysed to look for possible effects of nitrogen and fungicide application, as well as confirm the observation that seedrate was not an influential factor.

This second sample set consisted of 60 Optic barley samples, all grown at a single site with the range of seedrate, nitrogen and fungicide applications as detailed in the Methods. As before, all of the samples were malted and dried under identical conditions, and the folate content was then measured.

Statistical analysis revealed that nitrogen application, and not seed rate or fungicide application, had the most significant effect on the folate content of malts. The folate content in seeds grown at 150 kg N/ha were clearly higher than those grown at 50 and 100 kg N/ha (Figure 4).

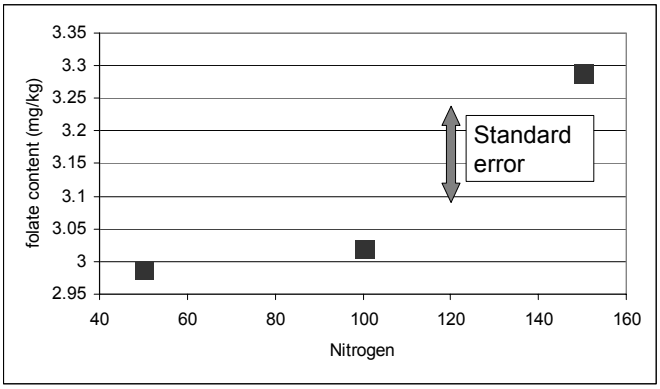

Figure 4. The relationship between nitrogen application and folate content in malt.
The malts were from Optic barley, grown at a single site at various N and fungicide applications and seedrate. The data for N application only are shown, together with an indication of a standard error of the grand mean.

The statistical analysis for an effect of nitrogen on the malt folate content gave an F.pr value of 0.08, which is a borderline positive result. For this reason, this result should ideally be followed up with another similar sample set to explore this phenomenon in more detail. From a biological standpoint however, it can be suggested that high nitrogen application could be beneficial in producing a seed that is 'well nourished'. In other words, the seed has formed under ideal conditions, and has been able to store the maximum amounts of nutrients, amino acids and biological cofactors which would assist a seed to have a rapid biosynthetic response during germination.

CONCLUSIONS

Determining the effect of parameters such as growth conditions and variety on a biological process such as vitamin synthesis is not simple to achieve. In this project, we have taken the approach of using a tightly defined sample set to answer some specific questions. From our data we are able to say that some varieties accumulate folates to a greater extent than others during germination, and that higher levels of nitrogen application may boost this accumulation. On the other hand, it is clear that we have had to limit our experiments to just a few variables in order to gain statistically significant data. For example, only 4 varieties and 3 growth conditions were analysed. Despite these limitations, this study gives barley growers and breeders some substantial information on how it may be possible to increase the nutritional value of malted cereals.

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