Reduction options for free asparagine and acrylamide in the wheat supply chain - cultivar selection, cropping, milling, puffing and baking

Prof. Dr. Friedrich Longin, State Plant Breeding Institute University of Hohenheim, Dr. Klaus Schwadorf and Dr. Ute Bertsche, Core Facility at University of Hohenheim, Dr. Andreas Baitinger, Miller school Im Hoppenlau, M.Sc. Matthias Rapp, W. v. Borries-Eckendorf GmbH & Co. KG, Dr. Lorenz Hartl, Bavarian State Research Center for Agriculture, Heiner Beck, Bakery BeckaBeck, Herman Gütler, Mill: Stelzenmühle, Kira Plake and Karsten Eisenhardt, H. & J. Brüggen KG; contact: friedrich.longin@uni-hohenheim.de

Summary

Acrylamide is a substance classified as carcinogenic that can be formed when starchy products are processed under heat. It is formed from reducing sugars and free asparagine via the Maillard reaction. Currently, the EU Commission works on threshold values for acrylamide in different products. Our aim was therefore to investigate how the acrylamide risk can be reduced along the wheat supply chain. To this end, we performed a series of different experimental studies. Firstly, we run large baking trials and were able to clearly demonstrate that the free asparagine content in whole grain flour correlates very closely with the acrylamide content in bread. A long dough fermentation time can significantly reduce the acrylamide content in bread. Secondly, we demonstrated that acrylamide content in puffed durum grains closely correlated with free asparagine content in the whole grain flour of the durum grains. Finally, we performed a series of experiments how free asparagine content can be influenced by breeding, fertilization, and milling. Thereby, the levels of free asparagine in the flour varied greatly between the cultivars within the species wheat, durum, spelt, emmer and einkorn. Interestingly, free asparagine contents of the cultivars did not correlate with other quality traits such as protein content, sedimentation value, grain size and falling number. Increased nitrogen fertilisation led to higher free asparagine contents, but the choice of cultivar had a greater influence on free asparagine contents than fertilisation. Finally for milling, the more of the outer layers of grains are used for flour production, the higher was the content of free asparagine. Consequently, there are numerous options available along the wheat supply value chain to sustainably reduce the acrylamide risk in grain products. It should be kept in mind, however, that only a quite small part of wheat products have acrylamide values higher than expected EU threshold values and, thus, only a small part of the wheat supply chain might need to work on acrylamide reduction strategies.

Introduction

Wheat (Triticum aestivum ssp. aestivum) is one of the most important staple foods worldwide and its daily consumption is part of a healthy and balanced diet according to leading world health organisations such as the WHO, FAO and EFSA, especially when wheat products are consumed as whole grain cultivars [cf. 1]. Acrylamide is a substance classified as carcinogenic that can be produced when starchy products such as potatoes and cereals are exposed to high temperatures [2, 3]. Deep-fried potato products in particular, such as crisps and chips, have high acrylamide concentrations, but acrylamide can also be detected in most cereal products, albeit in higher concentrations, especially in biscuits, gingerbread and crispbread [4, 5, 6]. In bread, over 95% of acrylamide is found in the crust and therefore hardly any acrylamide in the crumb [2]. The EU Commission lowered few years ago the "guideline value for acrylamide" in wheat bread to 50 µg/kg. For other breads it is 100 µg/kg, new regulations are expected soon.

In recent years, numerous methods have been tested to reduce the acrylamide content in end products. Acrylamide is formed under the influence of heat in the so-called Maillard reaction if sufficient reducing sugars and the amino acid free asparagine are present [2, 3, 7]. One of the most important measures to reduce acrylamide is to reduce the exposure to heat and thus avoid the Maillard reaction. However, it must be kept in mind that this Maillard reaction leads to the formation of a crispy and flavoursome crust, especially in bread. It is therefore important to find a compromise between minimising the effect of heat and ensuring sufficient proofing and flavour development. It has also been shown that the addition of the amino acid cysteine or the enzyme asparaginase can also significantly reduce acrylamide formation [2].

Another approach is to reduce the precursors of acrylamide already in the raw material, i.e. in the grain. As reducing sugars are present in abundance in the production of cereal products, it is important to influence the free asparagine content [8]. Several experimental field studies have shown for wheat that the sulphur supply of the soil has a significant influence on the amount of free aspargine in the grain [7, 9, 10, 11, 12, 13]. For example, a severe sulphur deficiency leads to greatly increased levels of free asparagine. In soils with a normal sulphur supply, as achieved by good agricultural practice with the recommended sulphur fertiliser applications, free asparagine levels were found to be significantly lower.

To our knowledge, there have been yet few studies on the question of whether cultivars differ in terms of their free asparagine content and, if so, how great this factor is in comparison to the environmental influence of the growing location or nitrogen fertilisation. In a simple laboratory study it was shown that a longer dough rest could reduce the amount of acrylamide [2], but a repeated study under real bakery conditions is still missing.

Material & Methods

We therefore started several trials in which numerous cultivars of wheat, durum, spelt, emmer and einkorn were grown at three locations each and then analysed in the laboratory for their free asparagine content in whole grain flour. In addition, numerous quality traits were determined in the laboratory in order to analyse their possible correlation with the free asparagine content. For einkorn, emmer and spelt, 10 cultivars each, for durum 25 cultivars and for wheat 149 cultivars each were grown at three locations in partially replicated trials. In the nitrogen fertilisation trial, seven wheat cultivars were grown at three locations in 2016/2017 at three nitrogen fertilisation levels (N1 = 175 kg nitrogen/ha, N2 = 200 kg nitrogen/ha, N3 = 230 kg nitrogen/ha, Nmin + fertilisation in each case). The relationship between free asparagine in whole grain flour and acrylamide in the bread crust was analysed in 20 different breads, which differed only in the wheat cultivar used, all baked with two different recipes. In each case, the wheat cultivar was a mixed sample from three growing locations and was milled into whole grain flour (Treffler Mühlomat, sieve perforation = 500 µm). The breads were baked once with a short and long dough fermentation. For the short dough fermentation, 2 kg whole grain flour, 40 g salt, 50 g yeast and 1.3 litres of water were processed into dough and baked after 1.5 hours fermentation time. For the long dough fermentation, the amount of yeast was halved and the amount of water increased to 1.5 litres, as is usual in a bakery. After kneading, the doughs were in the refrigerator for 17 hours and were baked at room temperature after a seven-hour acclimatisation period [for more details see 14]. The crude protein content was determined by NIRS (ICC Standard Method 159) and the free asparagine content was determined on the whole grain flour (EC No 152/2009, Annex III, Method F, for more details see [15]). The amount of acrylamide was determined on the crust of the 40 test breads according to [18].

In addition, the grain samples of five wheat cultivars from two growing locations were processed into standardised flours by experienced millers at a miller school and the free asparagine content was determined separately for bran, whole grain flour and the fine flours with ash content 1050 and 550. The Promylograph T was used as the basic milling machine. The coarse husk fractions were reground on a laboratory mill. Type 1050 was mixed from individual fractions.

For the puffing experiment, five winter durum cultivars relevant in German durum production were grown at three different locations, each with 0.1ha area per cultivar and location. From previous trials and practical agriculture it is known, that a reduction of nitrogen fertilization helps to reduce content of free asparagine. Thus, the cultivars were grown according to farmers best practice but without the late nitrogen fertilizer application. Durum grains were cleaned and polished before puffing with the respective machine used in routine puffing of breakfast cereals at H. & J. Brüggen KG. Acrylamide of puffed durum grains and free asparagine of whole grain durum flour were determined as described above. The statistical analysis was carried out with the statistical package R using the ASREML package.

The amount of acrylamide in the bread crust and of puffed grains correlates closely with the content of free asparagine in whole grain flours

Since the largest proportion of acrylamide in bread is found in the bread crust, we focussed on the bread crust when measuring acrylamide [e.g. 2]. The amount of acrylamide in the bread crust correlated with 0.89 with the free asparagine content of the initial flour when a short dough fermentation time was used (Fig. 3A). The correlation was slightly lower but still highly positive with 0.73 when a long dough fermentation time was used (Fig. 3B). This confirms other studies in the literature [e.g. 2, 3, 7, 11, 12] and shows that the amount of free asparagine in the flour plays a central role in the formation of acrylamide. The less it is in the grain, the lower the probability of increased acrylamide formation in the end product.

Depending on the used wheat cultivar, the amount of acrylamide varied between 72.0 and 399.2 µg/kg bread crust in the breads with the short dough fermentation time and between 48.8 and 250.8 µg/kg bread crust in the breads with the long dough fermentation time. This allows several conclusions to be drawn. Firstly, as we measured the amount of acrylamide not in the whole bread but in the bread crust and the bread crust is the smallest part of the bread, the amount of acrylamide in relation to the whole bread was far below the EU reference value in all tests. Secondly, the baker has the opportunity to significantly reduce the amount of acrylamide in the bread simply by extending the dough fermentation time, which is a "clean-label" method. And thirdly, the wide range of variation in the acrylamide values of the 40 test breads, which differed only in the wheat cultivar used and baked in short and long dough fermentation, shows the large effect that the choice of wheat cultivar can have on the amount of acrylamide in the end product.

In a second experiment, we investigated puffed grains from durum, where acrylamide is often close or even higher than future EU reference values for acrylamide content. Five cultivars of winter durum were grown at three different locations in Germany and free asparagine were determined on the whole grain flour. The harvested samples were then puffed on the large-scale puffing machine typical for cereal pop production at H. & J. Brüggen and analysed for acrylamide content. The five cultivars differed considerably in the amount of free asparagine and acrylamide (Fig. 4). Acrylamide content ranged from 100 µg/kg for the cultivar Diadur to 279 µg/kg for the cultivar Sambadur. Content of free asparagine ranged from 129.5 mg/kg for the cultivar Diadur to 272.1 mg/kg for the cultivar Sambadur. Moreover, the ranking and differences in free asparagine content of the respective whole grain flours were very similar to results for acrylamide content of the puffed grains. Thus, also for puffing the free asparagine content of whole grain flours can be used as a good predictor for acrylamide forming potential of a wheat batch. Based on that study, a stable teamplay between few farmers, a mill, and a breakfast cereal producer was established using the cultivar Diadur, grown with reduced nitrogen fertilization, traded, stored and finally puffed separately from the big durum supply chain based on contract supplies, which might be used as a bets practice example for future.

Cultivars differ significantly in their free asparagine content

Acrylamide is only formed under the influence of heat and is not present in freshly harvested grain. However, free asparagine can be measured in the grain and, as shown above, the free asparagine content correlates very closely with the amount of acrylamide in the end products. It therefore makes sense to analyse raw cereals for their free asparagine content. We were able to determine very different contents of free asparagine in the individual cultivars of all investigated wheat species (Table 1). In bread wheat, for example, the free asparagine content of the 149 cultivars analysed varied from 143.25 mg/kg to 453.34 mg/kg in whole grain flour. This was also shown by statistically significant genetic variances for the free asparagine content across all wheat cultivars. In this respect, it is theoretically possible to significantly reduce the amount of free asparagine in the raw material through cultivar selection. However, we also found that the free asparagine content of a cultivar differed considerably depending on the growing location, although the fertilisation and cultivation methods were the same at each location (Fig. 7). The growing locations from which millers receive their wheat supplies differ every year and the climate at the respective location differs even more, all of which influences the absorption of nutrients and, thus, the composition of the grain. In this respect, a targeted selection of the growing environment with the aim of reducing the content of free asparagine in grain is actually impossible. However, it is essential to ensure that the above-mentioned sulphur deficiency in the farmer field is avoided, which was taken into account in our trials.

Nitrogen fertilisation increases free asparagine content

In contrast to the random fluctuations of the climate at each location, nitrogen fertilisation is a variable that can be influenced by agriculture. We therefore cultivated and investigated seven wheat cultivars at three growing locations with three levels of nitrogen fertilisation. A clear trend emerged. The more nitrogen was fertilised, the higher the free asparagine content in the whole grain flour (Fig. 5). This confirms other recent studies in the literature [e.g. 16]. This effect is comparable to the protein content, which also increases with increasing nitrogen fertilisation [e.g. 17]. Reducing nitrogen fertilisation is therefore one way of lowering the levels of free asparagine and, thus, ultimately acrylamide in end products. However, in our view it is wrong to demand wheat with a low protein content as a matter of principle because of asparagine. Our studies clearly show that, without knowing the cultivar, it is not possible to draw conclusions about the expected free asparagine content from the protein content of a wheat sample alone. For example, of the 149 wheat cultivars analysed, some had a protein content of > 13% but < 200 mg/kg asparagine and, conversely, cultivars with a protein content of < 12% had > 300 mg/kg of free asparagine (Fig. 6). This is also impressively demonstrated by the fertilisation trial. Even at the highest fertilisation level, cultivar 4 had a lower asparagine content than cultivar 7 at the lowest fertilisation level. From the point of view of minimising free asparagine, it is therefore best to consider the interplay between cultivar and fertilisation (less nitrogen, sufficient sulphur). Furthermore, baking industry and international cereal trade ask for high protein contents and farmers get prize primes for that. Consequently, a potential reduction of nitrogen fertilisation must be managed with care warranting farmers interesting prizes for these special requests, which underlines the importance of teamplay along the whole supply chain.

Breeding for low free asparagine content possible, but necessary?

This brings us back to the possibility of selecting cultivars. Cultivars with a low free asparagine content are of course only accepted if they are good in other characteristics such as grain yield, disease resistance and baking quality. For this reason, we analysed the correlation between the free asparagine content and several quality criteria in wheat. As described above, the asparagine content of all cultivars hardly correlated with the protein content (Fig. 6, Tab. 1). There are cultivars that produce a lot of protein due to their genetics, but they do not necessarily have a lot of free asparagine. However, increases in nitrogen fertilisation lead to a higher protein and asparagine content in all cultivars (Fig. 5). The free asparagine content did not correlate with other important quality characteristics such as sedimentation value, falling number, grain size or, in the case of durum, with b-value and glassiness [15]. On the one hand, this means that the free asparagine content cannot simply be predicted on the basis of another quality trait, but must be measured itself. On the other hand, it also means that you could breed for cultivars with a low free asparagine content without having to expect negative consequences for other quality traits. However, breeding is a lengthy process and the analysis to determine asparagine is complex. In this respect, the question arises as to what extent the differences in cultivars shown are really important for the cereal industry and to what extent it is worthwhile to carry out costly breeding.

Asparagine is mainly found in darker flours

Together with the miller school Stuttgart, we analysed the asparagine content of flours from different cultivars and differentiated between whole grain flour, fine flour with ash content 1050 and extra fine flour with ash content 550 as well as remaining brans. For all cultivars and regardless of the place of cultivation, the bran contained by far the highest content of free asparagine (≥ 400 mg/kg, Fig. 7). In contrast, about half as much free asparagine was measured in the whole grain flour. Interestingly, the fine flour with ash content 1050 had almost as much free asparagine as the whole grain flour. In contrast, the extra fine flour with ash content 550 had only a very small amount of free asparagine (< 75 mg/kg), which confirms older studies [11]. Thus, the choice of flour type might help to reduce the free asparagine content. From this point of view, a flour with a low ash content is best, which is by far the most commonly used flour in bakeries. Interestingly, however, the free asparagine content of the different types of flour was also found to be dependent on the place of cultivation, but also on the cultivar. For example, the cultivar “Patras” stood out positively in this milling trial as well as in the large wheat cultivar screening (Tab. 2) and the fertilisation trial (Fig. 5, cultivar 4). This means that the content of free asparagine in the various flour fractions can at least be roughly estimated using a single measurement on whole grain flour.

However, we do not recommend using extra fine flour to avoid the acrylamide problem. Although it is by far the consumer's favourite, it has been proven beyond doubt that our daily bread is part of a healthy diet, albeit in the whole grain version. In this respect, other methods must be found for whole grain baking if acrylamide reductions are necessary.

Comparison of different wheat species

For this purpose, we determined the free asparagine content of numerous cultivars of the five wheat species wheat, durum, spelt, emmer and einkorn. The comparison between the species in our experiments is not completely independent of environmental variation, as the cultivation location and the year of cultivation differed in some cases for the individual species. However, the results are so clear that the following can be deduced. The cultivars of wheat, durum, spelt and emmer seem to have roughly the same range of variation in free asparagine content, while einkorn has about twice as much. One possible explanation is that einkorn has a significantly smaller grain compared to the other types of wheat analysed and therefore, in relative terms, more husk and less endosperm occurs in whole grain flour. However, according to our flour study, the free asparagine is mainly located in the outer layers, so the lower amount of asparagine in the other types of wheat is merely a dilution effect of the endosperm. This is also the reason why einkorn has significantly more positive ingredients such as minerals and vitamins than the other types of wheat. In this respect, possible acrylamide reductions are more important with einkorn than with the other species. Here too, the many positive ingredients of einkorn are so interesting that one should not avoid einkorn and especially whole grain einkorn from the outset because of acrylamide.

Conclusion

We cannot and do not want to use this study to answer the question of whether activities to reduce acrylamide in wheat products along the supply chain are necessary at all. Very few bakery products reach acrylamide levels such as French fries or crisps and all the test breads measured were well below the EU reference value for acrylamide. Our aim was to analyse various options along the wheat supply chain for their potential to reduce acrylamide. Firstly, we were able to confirm that the content of free asparagine in whole grain flour is very closely linked to the amount of acrylamide in end products. We were also able to clearly show that there are large differences in the free asparagine content of individual cultivars of wheat, durum, spelt, emmer and einkorn. This means that the acrylamide risk could be almost halved by cultivar selection. It would even be possible to further reduce the free asparagine content of wheat in the long term through targeted selection. Few post registration cultivar trials in Germany monitor and report therefore since few years the free asparagine content of tested wheat cultivars. Reduced nitrogen fertilisation also leads to reduced contents of free asparagine in flour, but is only recommended in combination with the choice of cultivar and taking baking quality into account. The baker can further reduce the acrylamide risk by extending the dough fermentation time. In addition, whole grain flour has significantly more free asparagine than fine flour. Other reduction options that have not been investigated by us include reducing the baking temperature and duration, using technical enzymes, and avoiding sulphur deficiency in the production of cereals. Consequently, there exist numerous options to sustainably reduce the acrylamide risk along the wheat supply chain. Nevertheless, a rough estimate for the German wheat market is that only 10% of the wheat grain produced in Germany might be used for end products, where acrylamide might get close to future EU reference values. It remains a question of teamplay across the supply chain, how to solve this jointly in future.

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Fig. 1: The breads of the 20 different cultivars, each baked with short (left, e.g. bread with no. 36 and the bread directly in front of it) and long dough fermentation (e.g. bread with no. 15 and the bread directly in front of it on the right; photo: BeckaBeck).

Fig 2: Polished durum before and after puffing

Fig. 3: Correlation between acrylamide in the bread crust and the content of free asparagine in whole grain flour in 20 test breads, each baked with a short and long dough fermentation time. The breads differed only in the wheat cultivar used, which represented a grain mixture from three growing locations.

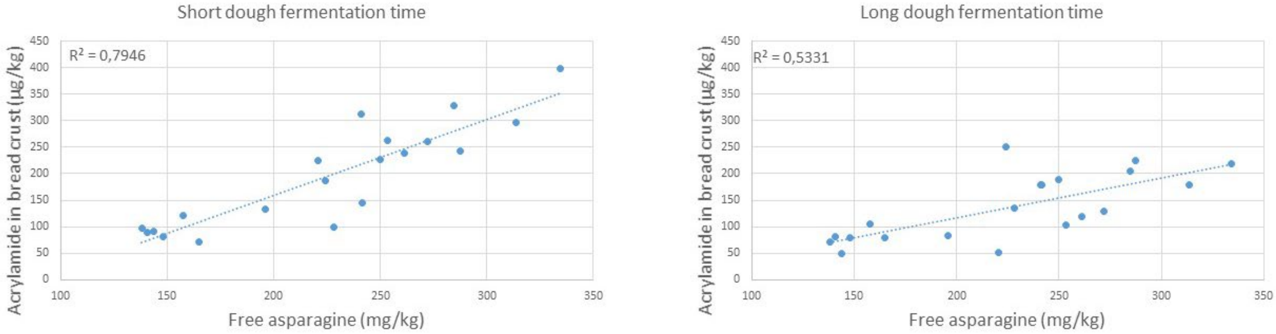


Fig. 4: Contents of acrylamide in puffed grains and free asparagine in whole grain flour from five different durum wheat cultivars (mean and standard deviation from 3 different test locations)

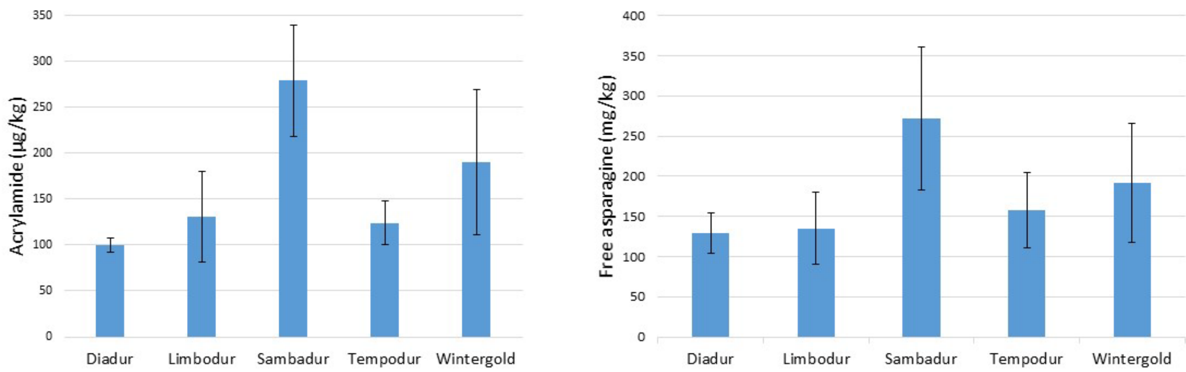


Fig. 5: Free asparagine content in whole grain flour of seven cultivars, each grown at three nitrogen fertilisation levels; each cultivar value is an average value from three growing locations (N1 = blue = 175 kg nitrogen/ha, N2 = orange = 200 kg nitrogen/ha, N3 = red = 230 kg nitrogen/ha)

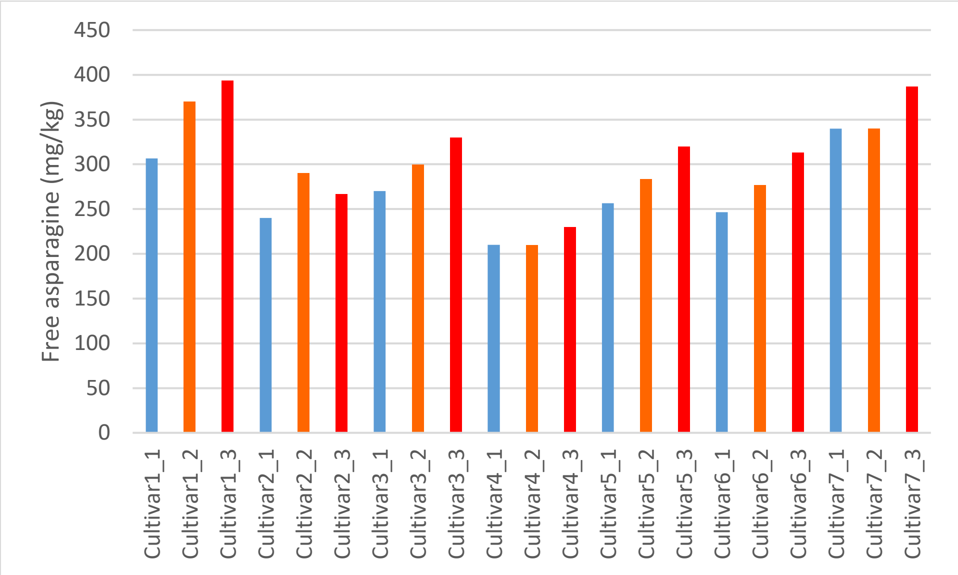


Fig. 6: Correlation between the contents of protein and free asparagine of 149 wheat cultivars; each cultivar value represents the mean value of three growing locations (DM = dry matter).

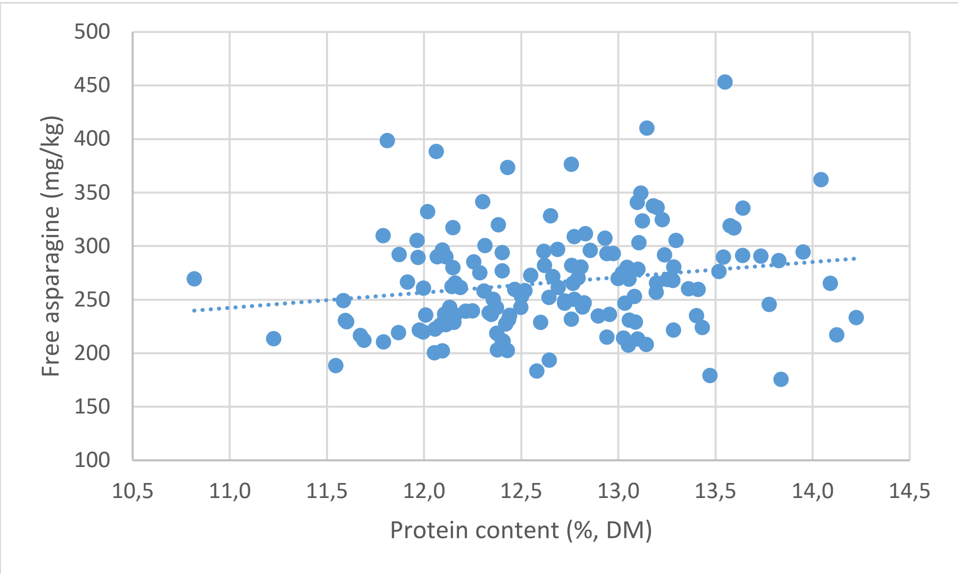
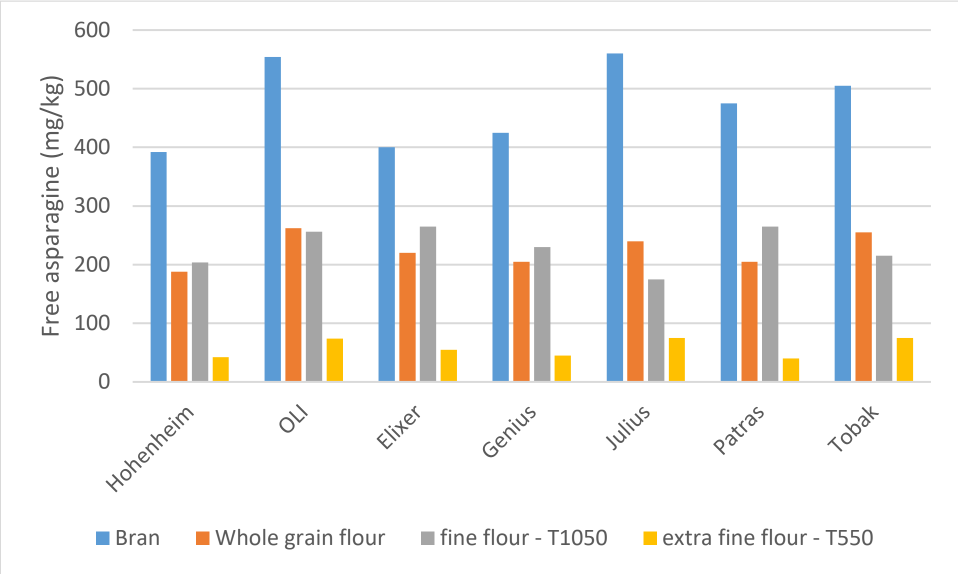


Fig. 7: Contents of free asparagine contents in different flour fractions; the mean values per location were calculated for the five wheat cultivars tested (Elixer, Genius, Julius, Patras, Tobak); the cultivar mean values were calculated from two growing locations (Hohenheim, Oberer Lindenhof (OLI)).



Tab.1 : Variance components for the content of free asparagine of whole grain flours in mg/kg (σ^2_G = genetic variance, σ^2_{GxL} = variance of the genotype x location interaction, σ^2_e = error variance), heritability (H^2), least significant difference at the 5% probability level (LSD), range of variation (minimum, mean, maximum value) and the correlation between protein and free asparagine content (r (PC, Asp)) of the cultivars averaged over three growing locations per species.

	Wheat	Durum	Spelt	Emmer	Einkorn
Min	143,25	161,96	210,06	239,97	551,28
Mean	241,69	270,25	307,67	308,2	711,13
Max	453,34	381,26	500,80	450,54	839,95
LSD5%	95,78	93,94	100,00	70,00	100,00
σ^2_G	0,004***	0,002***	0,0001	0,00003	0,00004
σ^2_{GxL}	0,004***	0,002***			
σ^2_e	0,003	0,002	0,00005	0,00002	0,00007
H^2	0,65	0,68	0,85	0,78	0,67
r (PC, Asp)	0,23**	-0,05	0,86***	0,4	-0,38

Tab.2 : Contents of free asparagine averaged across three growing locations for selected cultivars across five wheat species

Cultivar	Species	Free asparagine (mg/kg)
Oberkulmer Rotkorn	Spelt	245,75
Franckenkorn	Spelt	249,05
Badensonne	Spelt	255,68
Badenkrone	Spelt	298,1
Zollernspelz	Spelt	506,75
Bauländer Spelz	Spelt	546,64
Wintergold	Durum	194,9
Tempodur	Durum	230,72
Miradoux	Durum	286,93
Diadur	Durum	302,58
Sambadur	Durum	338,64
Terzino	Einkorn	693,02
Ramses	Emmer	266,62
Späth's Albjuwel	Emmer	283,63
Farvento	Emmer	347,13
Butaro	Wheat	179,41
Patras	Wheat	183,67
Discus	Wheat	203,16
JBAsano	Wheat	216,65
Bussard	Wheat	222,79
Colonia	Wheat	234,64
Akteur	Wheat	235,14
Apache	Wheat	235,93
Genius	Wheat	260,23
Julius	Wheat	280,17
Pionier	Wheat	280,43
Tobak	Wheat	341,76