

# Improving our knowledge of what becomes of alkaloids



*ARVALIS - Institut du végétal used its test bakehouse to measure the alkaloid content at different stages of the bread making process.*

**Traditional cleaning using a pre-cleaner does not totally exclude the presence of sclerotia. Ergots and their alkaloids can therefore find their way into the food production chain. ARVALIS - Institut du végétal measured the impact of some processes.**

According to scientific literature, the decontaminating effect of manufacturing processes varies greatly depending on the equipment used, the milling procedure and the type of product in question. The 50% reduction factor in the baking process is often mentioned for bakery products, but this figure can be much lower. Indeed, Fajardo (1995) does not identify any reduction at all in the soft central part of the bread through baking (25 minutes at 225°C), but does note a reduction of up to 55% in the crust.

For cookies baked for 17 minutes at 190°C, Merkel observes a reduction of up to 30% for some alkaloids.

Intent on ensuring the safety quality of the grain produced and processed, in 2012 and 2013, ARVALIS - Institut du végétal, ANMF (1) and IFBM - Qualtech undertook to study what becomes

of ergot sclerotia and alkaloids during various grain processing procedures. In addition, ARVALIS examined the influence of bread-making on alkaloids, and studied piglet growth.

## **Sclerotia behave similarly to grain**

The study of the impact of the milling process was carried out at the semi-industrial ENILIA-ENSMIC mill in Surgères (central western France), with financial support from FranceAgriMer. Two pure wheat varieties, produced in a farmer's field, were each divided into two batches of 450kg, and artificially contaminated with 0.5g/kg and 1g/kg of sclerotia, corresponding to a total alkaloid content around 850 µg/kg and 1,700 µg/kg respectively. Ten grain samples were analysed, as well as each flour stream, and all the co-products of the milled grain.

Results showed that sclerotia behave very similarly to grain when milled. They are broken up into pieces of varying sizes, then spread out according to their granulometric composition into all the milled products. Some research carried out abroad showed that sclerotia were flattened by the milling rollers. Sclerotia were therefore found in middlings. This was not the case in this study, but it does indeed happen during the crushing process (smooth rollers). Alkaloids are therefore heavily and naturally concentrated in fractions made up of coarser wheat middlings (2) and of wheat germ; the figures are on average respectively 14 times and 5 times higher than in the grain.

**During the milling trial, grain was cleaned to remove most of the impurities using ARVALIS - Institut du végétal's Plateforme Métiers du Grain (grain industry platform).**



### Estimates are still difficult to produce

In addition, the variability of the level of alkaloid from one sclerotium to another means that the sclerotium content of a batch is not a precise measure of the alkaloid content of the flour obtained through the milling process. Ergot contaminated wheat produces flour containing on average 25 to 45% less alkaloids than the grain itself. No reduction at all was noted in 25% of cases.

The stream factor can therefore be difficult to estimate, and even impossible to predict. Since alkaloids concentrate largely and naturally in co-products, particular care must be taken when using middling. At this stage, results need to be consolidated by testing other wheat varieties and the variation of other parameters.

Although the ENILIA-ENSMIC pilot mill is representative, results could be different in mills operating under real conditions.

*Claviceps purpurea* sclerotia are likely to contain 6 main alkaloids with an R-configuration (ergotamine, ergosine...), as well as the associated epimer forms with an S-configuration (ergotaminine, ergosinine...). The transition of each alkaloid from one configuration to the other is called epimerisation. This phenomenon can be reversed and does not follow any particular pattern. Those two forms also present different toxicological characteristics. It is therefore imperative to quantify each of the 12 alkaloids separately, and to define the total alkaloid content as the sum of those 12 characterisations.

### The bread-making process reduces the alkaloid content

The bread-making study carried out by ARVALIS in 2012 with the financial support of FranceAgriMer concentrated on a commercial grade flour. It established that having added 3.0 mg of ergot powder per gramme, the alkaloid concentration ranged from 0.06 mg/kg to 15 mg/kg.

The flour to dough transition saw the alkaloid content reduced by around 30% through the effect of dilution linked to flour hydration. Baking the dough then reduced it by a further 40%. This figure is similar regardless of the initial level of contamination in the flour. In this study, the whole process resulted in an overall reduction in alkaloids of 59% in the end product.

The different rate of alkaloids and of their epimer form, called R-form or S-form (see insert), showed that, in flour, the R-form is prevailing for all alkaloids. Conversely, the R/S ratio is variable depending on the alkaloid in question. The level of epimerisation increased during the process, primarily due to the effect of temperature.

All alkaloids were epimerised from the R-form to the S-form, with a minimal ratio between R- and S- forms in the crust. This information should be viewed in the context of the level of toxicity of those two forms, which, according to some research work, might be different.

### Improving our knowledge of the effect on animal feed

With the financial support of FranceAgriMer, ARVALIS established a trial on weaned piglets in the autumn of 2013. Its aim was to quantify the effect of two feeds with a total alkaloid contamination level of 2,350 and 5,050 µg per kg respectively (addition of 0.1 and 0.2% of ergot powder) on the piglets' performance, compared with an uncontaminated control feed. Occurrence studies carried out by ARVALIS in 2013 (data not published) show that the probability of reaching such levels of contamination in feed containing 50% of cereals is very low.

The quantification of the alkaloids contained in the ergot powder and the feeds indicates that the manufacturing process of feed (grinding, pelletisation at a temperature of 75°C...) did not alter their alkaloid content or their profiles.

In the study, all feeds were given as pellets and ad lib. to piglets between the age of 34 and 62 days, comprised of 36 castrates and 36 females (3 treatments x 24 repetitions). Two groups of piglets, all weaned at 21 days, were reared together in group pens and their growth was monitored over a period of 34 days. They were then lotted (3 groups of 12 animals, 6 males and 6 females per group) depending on their growth between 34 and 62 days of age.

The daily monitoring of the animals did not reveal any specific symptoms of ergotism such as balance problems or necrosis of their extremities.

« Particular care must be taken when using wheat middlings. »



**To date, very little growth performance data has been recorded for piglets receiving feed containing ergot alkaloids.**



**The alkaloid content is not reduced during feed manufacturing.**

### Performance is affected above 2000 µg/kg

From the start, the intake of contaminated feed was different from the intake of control feed. The difference in average daily intake (ADI) between the contaminated and uncontaminated feeds became greater after 2 weeks, to reach -10.7% and -18.4% respectively for each of the contaminated feeds. Throughout the 28 day trial, the ADI was lowest with the animals presented with the most contaminated feed (812 g/d, i.e. -17.8%). The low ADI of the most contaminated feed during the first two weeks resulted in low daily live weight gain (DLWG: 394 g/d), -21.6% lower than with the control feed (table 1).

After the 28 day trial, the DLWG of the animals receiving the most contaminated feed was significantly lower (by 15.7%) than the DLWG of the animals receiving the uncontaminated control feed (498 vs. 591 g/d). Growth performance with the least contaminated feed was in between, with 554 g/d.

The feed conversion ratios (quantity of feed consumed to produce 1 kg of live weight) are the same for all feeds, which shows that the nutritional value is not affected. Those results corroborate those obtained by Oresanya et al. (2003) that reported that piglets' growth was only affected if the ergot concentration in the feed reached 0.10%, which is the equivalent of a total alkaloid content of 2,070 µg/kg. Ergot alkaloids might be activating satiety mechanisms that in turn trigger a reduction in intake.

In addition, consumption of feed with a high alkaloid content (5,050 µg/kg of feed) resulted in a significant increase in white blood cells (+ 13%) and lymphocytes (+ 25%), indicating that the defence mechanisms of the immune system were stimulated. Conversely, an alkaloid content of about 2,300 µg/kg of feed does not seem to alter the piglets' blood profile.

(1) Association Nationale de la Meunerie Française (French Millers' Association).

(2) Milling co-product containing flour and fibre fractions.

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### The only applicable regulation concerns the presence of sclerotia

The detection of ergot in batches of cereals has been increasing since 2006. It is currently regulated when it reaches 1 g/kg of cereals in animal feed and 0.5 g/kg for wheat intervention batches. Reference documents\* also stipulate a maximum threshold of 0.5 g/kg of cereals for human consumption. But ergot sclerotia contain alkaloids. Even though the link between those two elements remains partly a grey area the toxins produced are steering Brussels towards regulating alkaloid content.

\*: Codex Alimentarius (CODEX STAN 199-1995) and Scientific and Technical opinion of the French Food Safety Agency (AFSSA) dated 3<sup>rd</sup> April 2008.