TECHNICAL PAPER

April 2019

EFFECTIVELY REDUCING ACRYLAMIDE BY MAXIMIZING ENZYMATIC ACTIVITY OF ASPARAGINASE

IN DIFFERENT APPLICATIONS

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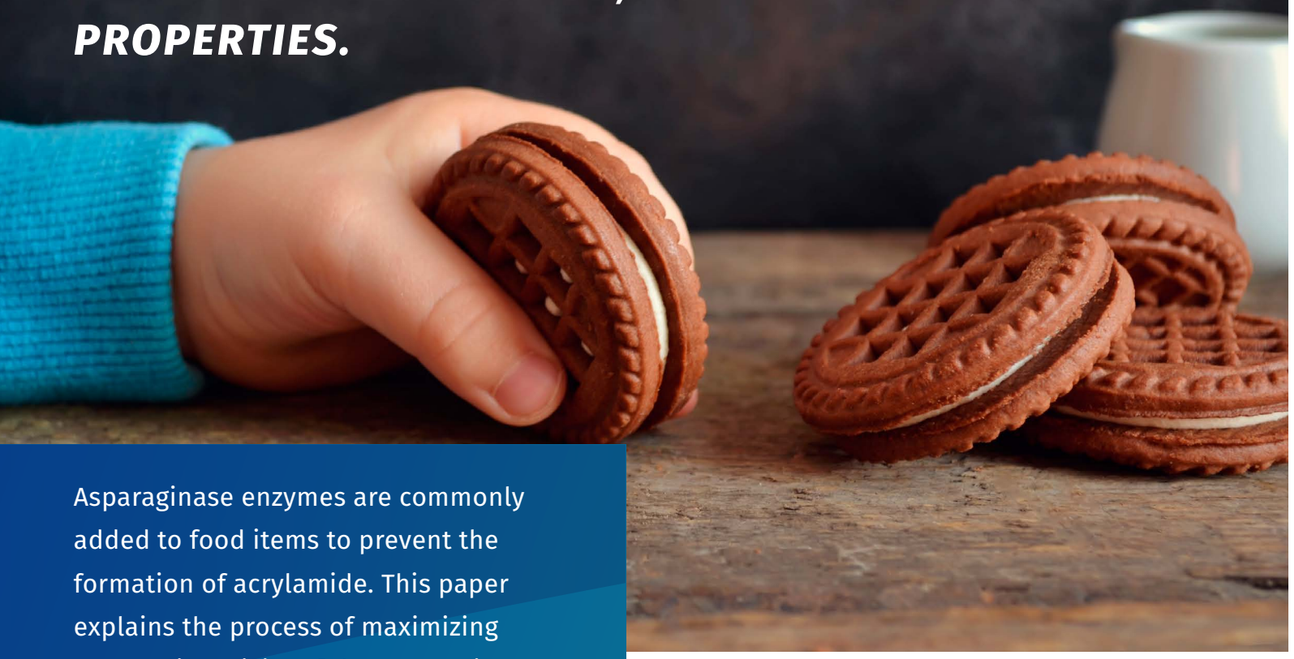
DSM

TECHNICAL PAPER

THE IMPORTANCE OF BALANCING WATER CONTENT, ASPARAGINASE CONCENTRATION, PRESENCE OF SUBSTRATE, AND MIXING PROPERTIES.



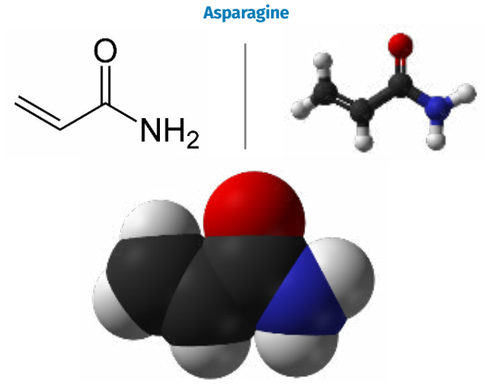
Asparaginase enzymes are commonly added to food items to prevent the formation of acrylamide. This paper explains the process of maximizing enzymatic activity of the asparaginase enzyme and reviews specific applications to explain the optimal combination of the presence of substrate, water content and the degree of mixing, for lowering acrylamide in baked goods. As concluded in this paper, the enzymatic activity of asparaginases is optimal in applications with a water content of 30% or higher but also in applications with lower water content, good enzymatic activity can be reached.

CHALLENGE FOR FOOD MANUFACTURERS

Consumers, manufacturers and the media are increasingly focusing on food safety and food safety incidents receive intense media attention. The formation of acrylamide in a variety of processed foods is part of this discussion and is a growing health concern. Generally, processed foods (like bread and biscuits, crackers, breakfast cereals, various snacks, and roasted products such as coffee) are mentioned as products to watch closely.

Acrylamide is formed when food containing reducing sugars is processed at high temperatures. Such processes include baking, frying, grilling, toasting and roasting, both in commercial-scale manufacturing of food products as well as in home cooking. Acrylamide formation is closely related to the desired browning effect; the Maillard reaction. This occurs between reducing sugars (such as glucose, fructose or lactose) and free amino acids. When the amino acid is free asparagine, a minor side-reaction occurs leading to the formation of acrylamide.

Asparagine



THE THREE FACTORS DETERMINING ENZYMATIC REACTION

Asparaginases are commonly added to food items to prevent the formation of acrylamide. Enzymes are biocatalysts that have to perform actions to be effective and need time to convert food components to products with improved properties.

Enzymatic action occurs in two steps. First, the enzyme needs to find its substrates (usually two: water and another substrate) and convert them into other product(s). Second, the enzyme needs to release these converted products so that it is ready to start the conversion of more substrates. Under optimal conditions, these steps are repeated until no more substrate is available.

From these considerations it becomes clear, that the **presence of substrate**, the **water content** and the **mixing regime** are the three factors that determine the speed of the enzymatic process.

1) If mixing is poor, the enzyme and substrates depend on passive diffusion to come together.
2) Also, if the water content is low, diffusion speed is also low and could limit the speed in the enzymatic process.
3) If the water content is exceptionally low, water is no longer a continuous phase, and the food matrix will break up into small water compartments in an otherwise dry matrix.

For an optimal enzymatic reaction, the enzyme, the water and the substrate need to be sufficiently present and be able to be in contact with each other – e.g. through efficient mixing. This must be the case in every so-called compartment. In an “empty” compartment, where there is no, or not enough, enzyme available, no conversion will take place.

The part of the substrate that resides in the “empty” compartment(s) will not be converted. Better mixing will improve the distribution and the presence of the enzyme throughout the dough. Of course, the right enzyme dosage is essential to make sure that each compartment contains sufficient enzyme. Dosage levels are outside the scope of this paper.

ASPARAGINASE ENZYME NEEDS A DISSOLVED SUBSTRATE TO BE ACTIVE

As mentioned earlier, asparaginase catalyzes the conversion of one amino acid – asparagine – and water to another amino acid – aspartic acid – and ammonia. This conversion prevents the formation of acrylamide. Asparaginase acts on a substrate (asparagine) dissolved in water. The other substrate is the water itself.
Concluding the above: for optimal enzyme activity the substrate must be in the water phase from the start. If the substrate instead is a dry ingredient instead of water, it must first be dissolved in the water phase so that the substrate can reach the active site of the enzyme.

SIZE AND MOBILITY OF ENZYMES MATTER

At the molecular level, enzymes are relatively large, consisting of hundreds of amino acid building blocks. In contrast, the substrate for asparaginase, asparagine, is much smaller, being just one amino acid. Smaller molecules can move much faster than larger molecules, especially under low-water conditions. Research has been conducted to discover whether the enzyme moves at all, by using an elegant microscopic technique called Fluorescence Recovery After Photobleaching (FRAP). A fluorescent marker is attached to the enzyme. After mixing the enzyme with the dough, a portion is spread out on a microscope slide, and at a certain spot the fluorescent marker is destroyed using a laser. This results in a bleached spot on the dough. If the enzyme is mobile, the fluorescence will return to the bleached spot, as the labelled enzyme moves in. However, if the enzyme is not mobile, the spot will remain bleached (Figure 2).
It was found that the bleached spot did not regain its fluorescence, leading to the conclusion that the enzyme is immobile in the dough system. Therefore, the mixing process serves mainly to extract asparagine from the flour into the water phase, and then to distribute it, dissolved in water, through the dough, where the enzyme is waiting.
Since acrylamide can occur in a wide range of baked goods, asparaginases are commonly used in a wide range of baked goods as well. But, as it becomes clear from table 1. different types of applications have diverging water contents and therefore require different mixing regimes.

Figure 2: The principle of FRAP

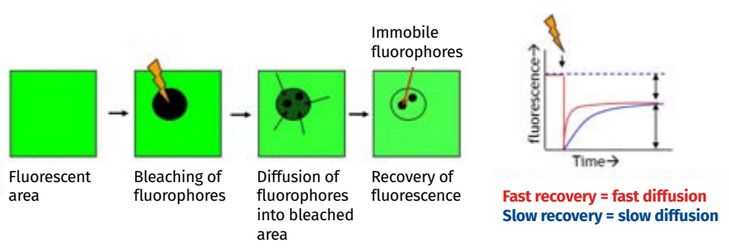


Figure 2. The principle of FRAP. A fluorescent area is imaged in time. A strong laser pulse irreversibly destroys all fluorophores in an area. Diffusion of fluorophores results in recovery of fluorescence over time. The graph on the right shows a plot of the fluorescence intensity of the bleached area as a function of time. The difference between initial intensity and final recovered intensity is due to immobilized bleached fluorophores. In short: no movement of the enzyme = no recovery of fluorescence.

Wet dough >30% moisture*	Typical degree of mixing
Bread	Intense to develop dough
Potato dough for snack	Relatively intense to intense
Tortilla/corn chips from masa dough	Relatively intense to intense to develop a sheetable dough
Infant cereals	Relatively intense
Crispbreads	Relatively intense
Pretzel snacks, crackers	Moderate

Dry products, 20-25% moisture*	Typical degree of mixing
Cookie dough (often fat rather than water as continuous phase)	Moderate to intense
Potato granules	Moderate to intense to blend homogenously
Breakfast cereals (extruded or pressure-cooked flakes)	Relatively intense to intense

Dry Product <20% moisture*	Typical degree of mixing
Cookie dough, for instance rotary mold	Moderate to intense

Table 1. Examples of mixing regimes and degrees for various applications. It contains example applications where asparaginase enzymes can be added but is not intended to be a complete list.
* Moisture levels are described from an enzymology perspective. Typically, 20-25% moisture in a mix or dough would be considered as a dry product (low moisture) and >30% would be considered as a wet dough (high moisture).

In the following chapters we explain the science behind the use of asparaginase for reduction of acrylamide in wet doughs - for applications such as bread, pretzels, crackers and masa dough -, in low-water content doughs, - for rotary mold cookies, potato granules and pellets -, in corn masa doughs - tortilla chips - and in cookie dough. In each application the relationship between water content, asparaginase enzyme concentration, substrate conversion and mixing properties are explained.

THE RELATIONSHIP BETWEEN ASPARAGINASE DOSAGE AND SUBSTRATE CONVERSION IN WET DOUGHS

In water-sufficient systems - with a water content >30% - a proportional relation exists between enzyme dosage and substrate conversion, this is illustrated in the graphs below that show corn masa and potato dough. Figure 3 shows the dose response of asparaginase in corn masa dough with relatively low asparagine content. Figure 4 shows a similar graph, but the subject is potato dough, with a high asparagine content, as substrate. Both graphs show the proportional relation between asparaginase dosage and substrate conversion at diverging asparagine levels: corn masa contains much less asparagine than potato flour.

PREVENTASE™ DOSE RESPONSE IN DOUGHS

Figure 3: Dose-response: corn masa

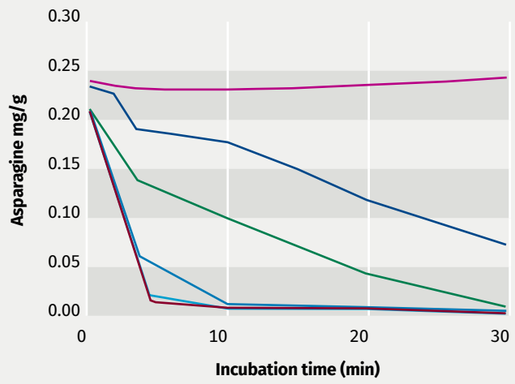
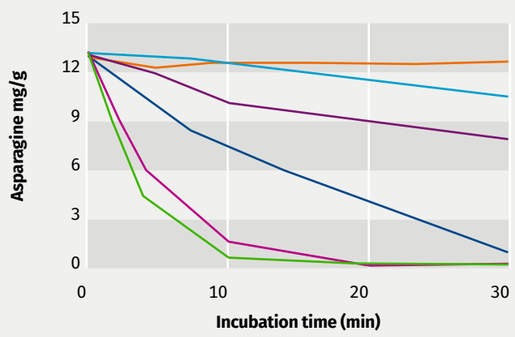


Figure 4: Dose-response: potato dough



Figures 3 & 4. The asparaginase response in wet doughs. Figure 3 shows the dose-response in corn masa dough (dosage levels from top to bottom: 0, 0.1, 0.2, 0.4, 1, 2 U/g flour). Figure 4 shows the dose-response in potato dough (dosage levels from top to bottom: 0, 1, 2, 5, 10, 15 U/g flour). At widely different absolute asparagine levels and enzyme dosage, the curves are very similar.

In wet doughs, high concentrations of asparagine can be converted quickly, but good conversion at low concentrations of asparagine can also be achieved. This implies that the enzyme has a high affinity for asparagine (required to work efficiently at low concentrations) but is not inhibited by high concentrations (required to be effective there too).

In a wet-dough it is relatively easy to add asparaginase effectively without any required adaptations to the recipe or production process. Moderate mixing is required, but this is often also needed to disperse and mix the dry ingredients in the water phase. Therefore, it is relatively easy to reduce acrylamide by adding asparaginase to bread, pretzels, cracker or masa dough. But, the wet slurry of infant nutrition cereals and the wet batter of crisp breads also offer optimal conditions for asparaginase.

THE EFFECT OF LOW WATER CONTENT IN POTATO GRANULES

In the manufacturing process of potato granules, the dry granules are mixed into the fresh potato mash to lower the water content and thereby increase the efficiency of the drying process. In this way, the end product, the granules, acts as a drying agent for the fresh potato, without the need for other ingredients. The water content of fresh potato is 75%, whereas the water content of the fresh/dry mixture is about 25%. This results in a crumbly mixture rather than a smooth dough, which posing a challenge for the enzymatic reaction.
Figure 5 shows how the asparagine content, in a 25% moisture potato dough, changes after treatment with the asparaginase enzyme. Fresh potato was mixed with dry potato granules at a ratio of 1:3 in a high-speed mixer. These are optimal mixing conditions (not representative of the industrial application) to discover if the enzyme can work under dry conditions. The mixture contained approximately 6 g/kg of asparagine, had a water content of about 25%, and was treated with various amounts of enzyme.

Figure 5: Change in asparagine content in a 25% potato dough

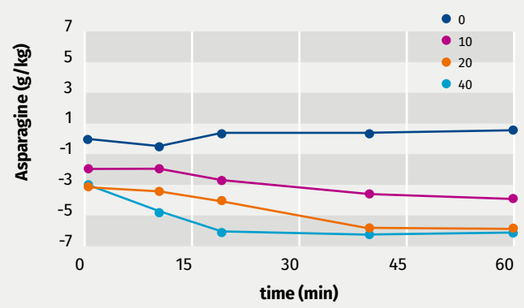


Figure 5. The change in asparagine content after treatment of a 25% moisture potato dough with asparaginase enzyme in different enzyme dosages. It is clear that also at 25% moisture, full conversion of asparagine can be achieved under these high-mixing conditions (enzyme dosage in U/g).

The above-mentioned trial describes the perfect situation. In reality, a potato granule production process is continuous and more complex. In each cycle, a limited amount of dry product is taken from the line (typically 20-30% of the total), to be replenished with freshly cooked potato. The mixture then passes through a series of mixers, and the moisture level is equilibrated before the mixture enters the drier.

This process poses challenges for analyzing the enzyme performance. Under steady-state production the dry product will be low in asparagine, and only the asparagine in the fresh potato needs to be converted by the enzyme. Since the process also removes enzymes with each cycle, the enzyme must be dosed in each cycle. However, during plant trials (or during start-up) the dry product is still high in asparagine, and, in the beginning, the enzyme concentration is low.

Figure 6 shows the amount of asparagine (ASN) and aspartic acid (ASP) during the potato granule process. The trial shows that it takes 20 hours to reach the steady state, when about 70% of the asparagine has been converted to aspartic acid. The results were analyzed using an iterative model to represent the successive cycles of the process. The model predicts that about 60% of the enzyme activity is carried over into the next cycle. It also predicts that the enzyme performs almost all its work in the mixers, and not during the moisture-equilibration that follows, even though that stage is significantly longer. This again illustrates the importance of mixing, particularly in such low-water systems, to achieve a good enzymatic conversion.

Figure 6: Levels of asparagine and aspartic acid as percentage of total measured amino acids

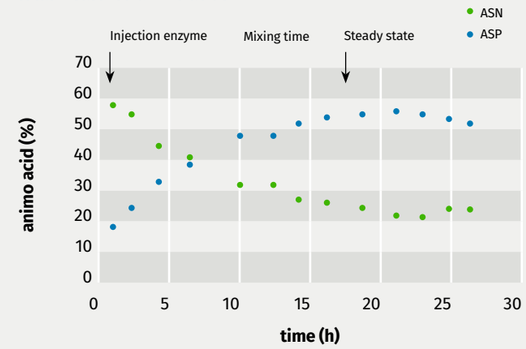


Figure 6. The amount of asparagine (ASN) and aspartic acid during the potato granule process. The concentrations of the amino acids are given as percentages of the sum of total measured amino acids (which is constant) to avoid variation due to the limited accuracy of sampling in a production plant. A gradual decrease of asparagine can be seen, and an increase of aspartic acid (ASP), until a steady-state situation is reached. About 70% reduction of asparagine has been achieved, showing that a significant reduction is possible in this dry process.

Figure 7A: Conversion of asparagine (ASN) at different water levels

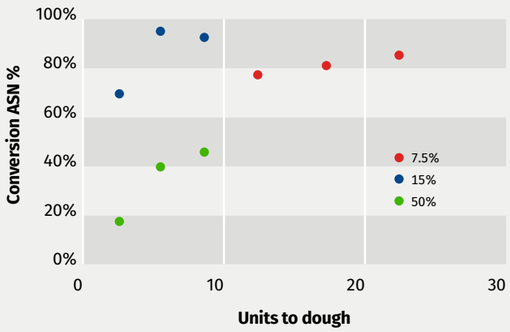


Figure 7A. The dose-response of asparaginase in doughs with different water percentage. At a higher water content (50%) the conversion of asparagine was less.

Figure 7B: Dough temperature after mixing at different water levels

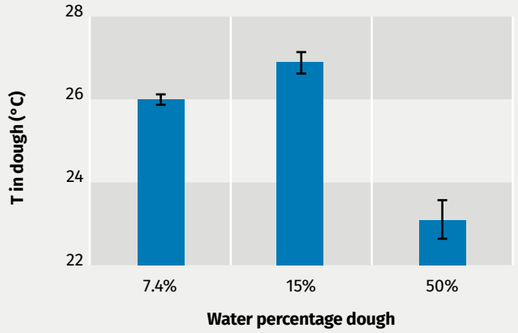


Figure 7B. The temperature of doughs with different water percentages directly after mixing. At a higher water content (50%) the temperature of the dough was significantly lower.

A SPECIAL CASE OF VERY LOW MOISTURE: COOKIE DOUGH

In general, a higher water content has multiple positive effects on enzyme activity, including improved extraction of asparagine, hydration of substrate and enzyme, and diffusion of asparagine, along with the creation of a continuous phase and improved mixing efficiency.

However, water content has multiple effects on a dough system. For instance, if dough processing takes place at ambient temperature, mixing can significantly increase the temperature. This is the case with cookie dough. Asparaginase works well at ambient temperature but will be more active at higher temperatures (up to about 70˚C). In the experiment shown in Figure 7, adding water made mixing so much easier that the temperature stayed low.

Figure 7A shows that the same amount of enzyme in the dough (here represented as “Units to dough”) gave a lower conversion of asparagine when the water content is 50%, compared to a water content of 15%. Figure 7B shows that this correlates with a lower temperature in the high-water content dough compared to the drier doughs. Although this is a rather special case, it illustrates that in applications many factors are connected, that simultaneously (but not independently) determine the efficiency of an enzymatic conversion. Nevertheless, improving mixing – by increasing power input, allowing more time, adding slightly more water, increasing the temperature, etc. – nearly always leads to a better result.

Ready to discuss your acrylamide challenges with us?
Contact us via **dsm.preventASe@dsm.com**

RECOMMENDATIONS OF TYPE OF PREVENT ASE® AND STARTING DOSAGE

Product category	Product	PreventASe® L/M/W*	PreventASe® XR	Recommended Starting Dosage	Estimated Reduction
Savory Snacks	Tortilla/Com Chips		X	50 ppm on maize flour weight	Up to 85%
	Pretzels/Salty sticks	X	X	20-120 ppm on flour weight	Up to 80%
	Pellets (Potato)	X		1000 ppm on dried potato weight	Up to 70%
Baked Goods	Bread & Cookies	X	X	100-500 ppm on flour weight	Up to 80%
	Bread & Other Fermented Goods	X		20-50 ppm on flour weight	Up to 90%
	Bread Substitutes	X		20-50 ppm on flour weight	Up to 80%
	Savory/Snack Crackers	X	X	50-100 ppm on flour weight	Up to 80%
	Specialty Goods	X	X	20-200 ppm on flour weight	Up to 95%
Infant Nutrition	Infant Cereals	X		100-500 ppm on flour weight	Up to 80%
	Infant/Baby Biscuits	X		100-500 ppm on flour weight	Up to 60%
Other	Breakfest Cereals	X		>150 pp, on cereal mix weight (extruded & toasted wheat flakes)	Up to 84%
	French fries	X		200-2000 ppm depending on process	Up to 60%
	Dried potato Products	X		100-600 ppm	Up to 80%

Table 2. Recommendations of type of PreventASe® asparaginase enzyme and starting dosage.

*L (liquid), M (granulate for non-wheat based products), W (granulate for wheat based products)

CONCLUSION

Acrylamide, which is formed when food containing reducing sugars is processed at high temperatures, is associated with health and food safety concerns. Asparaginase enzymes are commonly added to food items to prevent the formation of acrylamide. To maximize the effect of asparaginase enzymes, the presence of substrate, the water content and the degree of mixing need to be balanced.

In general, enzyme activity and mixing are optimal when the water content is around 30% or higher, which is the case in applications such as bread, pretzels, crackers, potato dough for snacks and corn masa dough for tortillas. In these wet-dough applications it is relatively easy to add asparaginase effectively without the need to adapt the recipe or production process. The wet slurry of infant cereals and the wet batter of crisp breads also offer optimal conditions for effective asparaginase use.

In systems with 20-25% water - such as cookie dough and potato granules - it is still possible to achieve a good enzymatic conversion, but special care must be taken with respect to mixing regime, and the way the enzyme is added to the food matrix. A dry cookie dough with intense mixing is a special case due to the interplay of many connected factors that simultaneously (but not independently) determine the efficiency of enzymatic conversion. Nevertheless, improving mixing – by increasing power input, allowing more time, adding slightly more water and increasing the temperature – nearly always leads to a better result.

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