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Mitigation of acrylamide by asparaginase in bakery products with different dough types and properties

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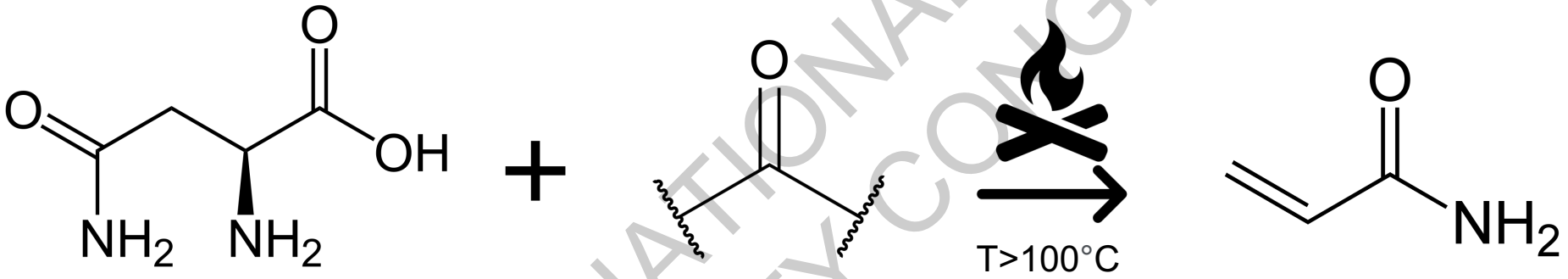
Acrylamide

Maillard reaction



Asparagine | Carbonyl groups | Acrylamide

IARC Group 2A

'acrylamide is probably carcinogenic to humans'

Acrylamide in Bakery Products

European Food Safety Authority

EFSA Journal 2012;10(10):2938

SCIENTIFIC REPORT OF EFSA

Update on acrylamide levels in food from monitoring years 2007 to 2010 [1]

European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

Food category	n	Median (µg/kg)	Mean (µg/kg)	P90 (µg/kg)	P95 (µg/kg)	Maximum (µg/kg)
Soft bread	176	25 (20-41)	75 (68-82)	169	310	1 778
Unspecified bread	4	716	1044	2 565	2 565	2 565
Breakfast cereals	144	84 (84-100)	149 (140-158)	333	420	1 600
Biscuits, crackers, crisp bread and similar	938	183	326(324-328)	837	1 235	4 200
Crackers	27	135	237 (236-238)	755	900	1 526
Crisp bread	198	117	232 (229-235)	480	765	2 430
Wafers	33	128	230 (229-232)	478	694	1 378
Ginger bread	458	209	387 (386-388)	1 074	1 372	3 615
Other biscuits, crackers, crisp bread and similar	222	189	309 (306-311)	672	1 001	4 200

'ALARA' approach

Benchmark level 350 µg/kg

High asparagine content of cereal flours!!

Acrylamide Reduction Strategies

Using asparaginase is a very efficient strateg
having long resting or leavening time. It com
ACR reduction rate and low impact on senso
In some products the enzyme has no time to
thus the application will not be effective. In ac
bakery formulations do not provide
for asparaginase action
Avoiding

Food & Function

PAPER

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Acrylamide mitigation strategies: critical appraisal of the FoodDrinkEurope toolbox†

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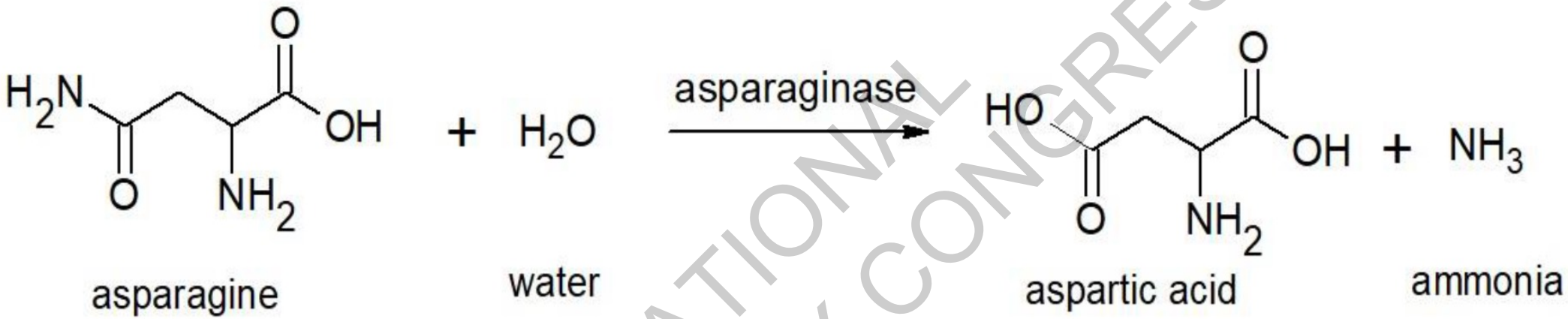
FoodDrinkEurope Federation recently released the latest version of the Acrylamide Toolbox to suppo
manufacturers in acrylamide reduction activities giving indication about the possible mitigation strategie
The Toolbox is intended for small and medium size enterprises with limited R&D resources, however
comments about the pro and cons of the different measures were provided to advise the potential use
Experts of the field are aware that not all the strategies proposed have equal value in terms of efficacy a
cost/benefit ratio. This consideration prompted us to provide a qualitative science-based ranking of t
mitigation strategies proposed in the acrylamide Toolbox, focusing on bakery and fried potato produc
Five authors from different geographical areas having a publication record on acrylamide mitigation stra
egies worked independently ranking the efficacy of the acrylamide mitigation strategies taking in
account three key parameters: (i) reduction rate; (ii) side effects; and (iii) applicability and econom
impact. On the basis of their own experience and considering selected literature of the last ten years, t
authors scored for each key parameter the acrylamide mitigation strategies proposed in the Toolbox.



Asparaginase for Mitigation of Acrylamide

One of the most promising acrylamide mitigation strategies

Effectiveness depends on pH, water activity, temperature etc.

The aim of the study

- Testing the effectiveness of asparaginase in acrylamide mitigation
- in the dough of different bakery products
- by changing the processing conditions
- without affecting the quality parameters of the products

Experimental Design

Bakery products

Rotary molded biscuit dough
Wire cut cookie dough

Creaming method

Rotary cut biscuit dough
Rotary cut cracker dough
Wafer batter

All in one method

Dough shaping

pH
water activity
asparagine

Baking
(210-230 °C for 8-9 min)

Cooling

spread ratio
color
acrylamide

Processing parameters tested

Enzyme dosage (1000-12000 ASNU)
Addition of dough resting period (RT)
Dough resting time & temperature (15-30 min, 25-37°C)
Mixing speed & mixing time (MS x MT)
Change in the mixing procedure (MPC)

Results

Bakery Products	ASNU Applications*	Acrylamide (µg/kg)	Acrylamide reduction (%)	Asparagine (mg/kg)	Asparagine reduction (%)
Rotary cut biscuit dough a_w 0.85 pH 8.50	Control	1672 ± 74^{a}	–	122.7 ± 12^{a}	–
	1000 ASNU	1086 ± 29^{b}	35	40.2 ± 7.3^{b}	68
	2000 ASNU	588 ± 32^{de}	65	6.6 ± 1.8^{c}	95
	3000 ASNU	330 ± 42^{f}	80	1.5 ± 0.4^{c}	99
	1000 ASNU + 15 min RT	933 ± 2^{bc}	44	19.2 ± 1.6^{bc}	85
	2000 ASNU + 15 min RT	398 ± 42^{ef}	76	2.1 ± 0.3^{c}	98
	3000 ASNU + 15 min RT	67 ± 16^{g}	96	0.2 ± 0.3^{c}	100
	2000 ASNU + 30 min RT	404 ± 47^{ef}	76	4.9 ± 0.3^{c}	96
	2000 ASNU + 15 min × 37 °C RT	262 ± 103^{fg}	84	1.1 ± 0.6^{c}	99
	2000 ASNU + MS	790 ± 76^{cd}	53	10 ± 0.1^{c}	92
	2000 ASNU + MT	720 ± 16^{cd}	57	7.3 ± 2.7^{c}	94
	2000 ASNU + MS × MT	774 ± 126^{cd}	54	5.6 ± 1.3^{c}	96
	2000 ASNU + S	820 ± 14^{cd}	51	13.8 ± 2.5^{c}	89

*ASNU: Amount of L-asparaginase that syntheses one micromole of ammonia per minute under standard conditions per kg flour.
S: Shortening, RT: Resting Time, MS: Mixing Speed, MT: Mixing Time, MPC: Mixing Procedure Change. Uppercase letter indicates the statistically significant differences ($p < 0.05$) in the columns for each biscuit type according to Tukey's test.

- 96% acrylamide reduction
- Increasing enzyme concentration (effective)
- Addition of 15 min resting time (effective but less)

Results

Bakery Products	ASNU Applications*	Acrylamide (μg/kg)	Acrylamide reduction (%)	Asparagine (mg/kg)	Asparagine reduction (%)
Rotary molded biscuit dough a_w 0.70 pH 7.55	Control	423 ± 71[a]	–	113.5 ± 2.3[ab]	–
	2000 ASNU	399 ± 22[a]	6	113.3 ± 4.5[ab]	–
	2000 ASNU + 15 min RT	438 ± 9[a]	–	113.8 ± 2.1[ab]	–
	2000 ASNU + 15 min × 37 °C RT	496 ± 5[a]	–	103.0 ± 13.2[abc]	9
	2000 ASNU + $NaHCO_3$	479 ± 132[a]	–	117.2 ± 5.5[a]	–
	2000 ASNU + MPC	412 ± 109[a]	3	87.6 ± 2.1[c]	23
	5000 ASNU + 30 min RT	500 ± 75[a]	–	90.4 ± 3.2[bc]	20

*ASNU: Amount of L-asparaginase that syntheses one micromole of ammonia per minute under standard conditions per kg flour.
S: Shortening, RT: Resting Time, MS: Mixing Speed, MT: Mixing Time, MPC: Mixing Procedure Change. Uppercase letter indicates the statistically significant differences ($p < 0.05$) in the columns for each biscuit type according to Tukey's test.

- Any of the applications are effective

Results

Wire cut cookie dough

a_w 0.74
pH 7.47

Bakery Products	ASNU Applications*	Acrylamide (µg/kg)	Acrylamide reduction (%)	Asparagine (mg/kg)	Asparagine reduction (%)
	Control	884 ± 118^{ab}	–	129.3 ± 10.3^{a}	–
	1000 ASNU	872 ± 31^{ab}	1	116.2 ± 1.2^{ab}	10
	2000 ASNU	935 ± 127^{a}	–	105.6 ± 4.3^{abc}	18
	3000 ASNU	797 ± 26^{abc}	10	102.0 ± 7.2^{abcd}	21
	5000 ASNU	616 ± 104^{bcdef}	30	100.9 ± 12.7^{abcd}	22
	7000 ASNU	528 ± 36^{cdef}	40	90.3 ± 4.4^{bcde}	30
	9000 ASNU	623 ± 73^{bcdef}	30	87.0 ± 2.4^{bcde}	33
	12000 ASNU	557 ± 11^{cdef}	37	90.6 ± 14.4^{bcde}	30
	1000 ASNU + 15 min RT	862 ± 130^{ab}	2	116.9 ± 1.6^{ab}	9
	2000 ASNU + 15 min RT	774 ± 34^{abcd}	12	101.9 ± 1.4^{abcd}	21
	2000 ASNU + 30 min RT	712 ± 0.1^{abcde}	19	96.0 ± 0.9^{abcd}	26
	3000 ASNU + 15 min RT	657 ± 27^{bcdef}	26	104.1 ± 9.8^{abc}	19
	5000 ASNU + 15 min RT	542 ± 29^{cdef}	39	78.7 ± 13.7^{cde}	39
	7000 ASNU + 15 min RT	469 ± 38^{ef}	47	73.3 ± 10.8^{cde}	43
	9000 ASNU + 15 min RT	480 ± 82^{ef}	46	68.5 ± 3.2^{de}	47
	9000 ASNU + 15 min × 37 °C RT	443 ± 50^{ef}	50	57.2 ± 11.4^{e}	56
	12000 ASNU + 15 min RT	505 ± 9^{def}	43	72.5 ± 8.6^{cde}	44
	2000 ASNU + MPC	552 ± 29^{cdef}	38	104.9 ± 1.0^{abc}	19
	5000 ASNU + MPC	406 ± 20^{f}	54	88.3 ± 12.1^{bcde}	32
	9000 ASNU + MPC	515 ± 8^{def}	42	97.7 ± 4.9^{abcd}	24

*ASNU: Amount of L-asparaginase that syntheses one micromole of ammonia per minute under standard conditions per kg flour.
S: Shortening, RT: Resting Time, MS: Mixing Speed, MT: Mixing Time, MPC: Mixing Procedure Change. Uppercase letter indicates the statistically significant differences ($p < 0.05$) in the columns for each biscuit type according to Tukey's test.

- 54% acrylamide reduction
- Increasing enzyme concentration (effective)
- Addition of 15 min resting time or change in mixing procedure (not significant)

Results

Bakery Products	ASNU Applications*	Acrylamide (μg/kg)	Acrylamide reduction (%)	Asparagine (mg/kg)	Asparagine reduction (%)
Rotary cut cracker dough a_w 0.92 pH 7.91	Control	1377 ± 129^{a}	–	126.1 ± 8.2^{a}	–
	2000 ASNU	757 ± 173^{b}	45	26.2 ± 11.9^{b}	79
	3000 ASNU	288 ± 76^{c}	79	12.0 ± 5.8^{b}	90
	2000 ASNU + 15 min RT	458 ± 122^{bc}	67	24.1 ± 0.5^{b}	81
	3000 ASNU + 15 min RT	279 ± 45^{c}	80	8.5 ± 1.5^{b}	93
	2000 ASNU + 15 min × 37 °C RT	491 ± 72^{bc}	64	16.2 ± 6.2^{b}	87
Wafer batter a_w 0.97 pH 6.99	Control	–	–	137.0 ± 5.9^{a}	–
	1000 ASNU	–	–	4.7 ± 1.3^{b}	97
	2000 ASNU	–	–	6.4 ± 0.6^{b}	95
	1000 ASNU + 15 min RT	–	–	6.0 ± 0.3^{b}	96
	2000 ASNU + 15 min RT	–	–	5.6 ± 0.4^{b}	96
	2000 ASNU + 30 min RT	–	–	5.6 ± 1.0^{b}	96
	1000 ASNU + 15 min × 37 °C RT	–	–	4.8 ± 0.1^{b}	96

*ASNU: Amount of L-asparaginase that syntheses one micromole of ammonia per minute under standard conditions per kg flour.
RT: Resting Time. Uppercase letter indicates the statistically significant differences ($p < 0.05$) in the columns according to Tukey's test.

- 80% acrylamide mitigation in rotary cut cracker dough
- Increased enzyme concentration (effective)
- 97% asparagine reduction in wafer batter

Results

No significant change ($p>0.05$) in L*a*b* values & spread ratio

Bakery product	The most effective enzyme application
Rotary cut biscuit dough baked at 230 °C for 8.5 min	
Control 	3000 ASNU + 15 min RT  100% asparagine reduction 96% acrylamide reduction
Wire cut cookie dough baked at 210 °C for 8 min	
Control 	5000 ASNU + MOC  32% asparagine reduction 54% acrylamide reduction
Rotary cut cracker dough baked at 210 °C for 9 min	
Control 	3000 ASNU + 15 min RT  93% asparagine reduction 80% acrylamide reduction

Conclusion

Acrylamide mitigation of 54-96% is possible.

Increasing enzyme dosage and applying 15 min of dough resting are the most effective applications.

a_w>0.75 is required for an effective acrylamide mitigation.

No change in quality characteristics.

Asparaginase application is promising for bakery products depending on the type of the product.

For further information & details…

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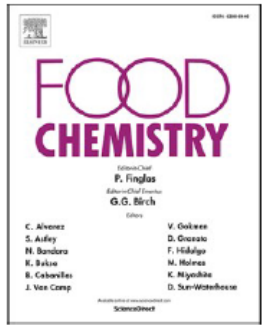

Effectiveness of asparaginase on reducing acrylamide formation in bakery products according to their dough type and properties

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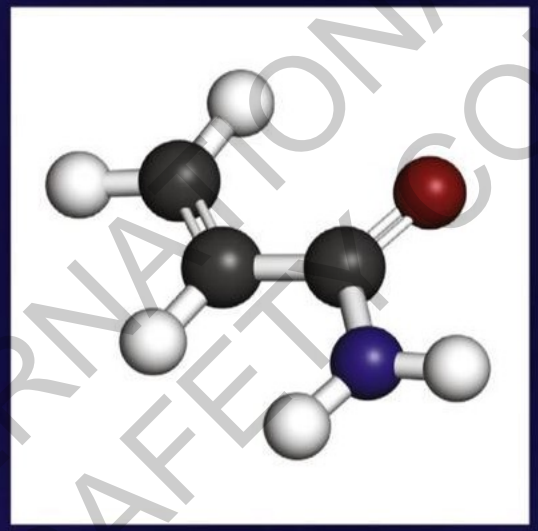
Acrylamide in Food

Analysis, Content & Potential Health Effects



Edited by **Vural Gökmen**

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THANK YOU

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