Mustard: A Natural Antimicrobial Ingredient

Did you know?

- Mustard has natural antimicrobial properties, the bioactive compounds - glucosinolates in mustard, are converted to the antimicrobial isothiocyanates in the presence of water
- Natural preservative functionality of mustard can be very valuable to the food industry
- Mustard isothiocyanates can effect up to a 5-log reduction of *E. coli* 0157:H7 in fermented meats
- Mustard Essential Oils (MEO) can be added to bakery products to inhibit fungal growth and production of aflatoxins
- Glucosinolates from deheated / deodorized (bland) mustard can be converted into highly antimicrobial isothiocyanate by bacterial myrosinase-like enzyme action present in *E. coli, 0157:H7*, *Staphylococcus carnosus* and *Pediococcus pentosaceus*[11,12,13] and in *L. monocytogenes*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Salmonella typhimurium*

Mustard's inherent antimicrobial properties should fit well with the food industry's growing interest and increasing consumer demand for the use of a natural preservative to enhance food safety and increase shelf-life of prepared packaged foods with a "clean label" claim.

Mustards in Foods

Mustards (Yellow and Brown) are commercially available as whole seeds, ground/cracked seeds, meals or flour forms and are widely used in the manufacture of condiments, salad dressings, pickles, sauces, processed meats and as substitutes for egg ingredients. While mainly used as a spice or for its functional properties, mustard can also provide raw and processed foods protection against pathogenic and spoilage microorganisms.

Antimicrobial Bioactives in Mustard

All mustards, Yellow (& White) (*Sinapis alba*) and Brown/Oriental (*Brassica juncea*), contain glucosinolates. It is these glucosinolates and their isothiocyanate (ITC) breakdown products which contribute to its natural antimicrobial activity and to the heat and pungency of mustard.

The main glucosinolate in Brown/Oriental mustards is **sinigrin** which when hydrolyzed by the inherent enzyme myrosinase in the presence of water, yields volatile *allyl isothiocyanate* (AITC) which has a sharp taste and pungent aroma. Hydrolysis of the glucosinolate **sinalbin** in Yellow/White mustard yields *para–hydroxybenzyl isothiocyanate* (p-HBITC) which is responsible for the hot mouthfeel (Table 1).

Thermal processing of mustard inactivates the myrosinase enzyme, thus preventing glucosinolate hydrolysis and isothiocyanate formation and results in bland flavoured mustards more suited for incorporation into many foods.

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De-heated (cold, deodorized) mustard:
Mustard is subjected to a heat treatment which deactivates the myrosinase enzyme leaving the glucosinolates intact. Flavour is usually mild.

Regular (hot) mustard:
No heat treatment; mustard retains its heat (hot taste) and spiciness. The myrosinase enzyme is active and in the presence of water converts the glucosinolates to isothiocyanates.

ITCs are present in mustard as part of the mustard essential oil (MEO) component in whole seed, ground seed, flours and meals. The MEO containing AITC can be extracted from Brown/Oriental mustard and used 'as is' in food products. AITC from natural sources is permitted as a food preservative in Japan and as a GRAS (Generally Recognized as Safe) flavouring agent in the United States.[5] White mustard essential oil (WMEO) containing p-HBITC also has the potential to be commercialized as an antimicrobial agent complementing, but not replacing, existing process controls.[6]

Table 1. Type of glucosinolate and isothiocyanate present in Yellow and Brown/Oriental mustards

Mustard	Glucosinolate	Content in Seed	Isothiocyanate
Yellow (*Sinapis alba*)	Sinalbin	2.3%	p-HBITC
Brown and Oriental (*Brassica juncea*)	Sinigrin	0.8%	AITC

Isothiocyanates, and particularly purified AITC, have been widely studied as antimicrobial agents and show a wide antimicrobial spectrum inhibiting Gram-positive and Gram-negative bacteria, yeasts and mould (Table 2). AITC is antimicrobial in both liquid and gas forms, but gaseous AITC has greater antimicrobial potency.

Table 2. *In vitro* test of AITC on agar surfaces containing microorganisms [3,4,17,20]

AITC vapour tested on agar surfaces		
Microorganism	**Temperature (°C)**	**MIC* (µg/L)**
Bacteria		
***Escherichia coli* 0157:H7**	37-40	34-1500
Listeria monocytogenes	40	1500
***Salmonella* spp.**	37-40	54-1500
***Pseudomonas* spp.**	5-37	54-1500
Mould		
***Alternaria* spp.**	25	22
***Aspergillus* spp.**	5 - 25	37-100
***Botrytis* spp.**	5 - 25	100
***Fusarium* spp.**	25	16-34
***Penicillium* spp.**	25	22-62
***Minimum inhibitory concentration (µL/L is ppm or parts per million)**		

WMEO is more effective against pathogenic and spoilage Gram-negative bacteria (*Escherichia coli*, *Salmonella enterica, Enterobacter aerogenes*) than Gram-positive bacteria (*Listeria monocytogenes*, *Bacillus cereus* and *Lactobacillus fermentum*) with its activity being strain specific.[14] In addition, p-HBITC also showed significant bactericidal activity against other bacteria including *Staphylococcus aureus*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*, *Shigella boydii* and *Clostridium perfringens* in media[7] and poultry.[15]

Mode of Action

AITC has a multi-targeted mechanism of action that causes membrane damage and enzymatic inhibition.[2,10,11] Little, as yet, is known about the specific mode of action of p-HBITC from Yellow mustard.

Supporting Research

Recent research has shown that Yellow mustard flour can be used as a natural alternative to eliminate *E. coli* O157:H7 from dry fermented sausage or other meats (Table 3). The goal is a 5-log reduction of *E. coli* viability during manufacture of fermented sausages as required by North American regulatory agencies. Cooking of fermented meat products is effective but reduces the value and alters the sensory characteristics of the sausage.[8]

Table 3. Reduction of *E. coli* 0157: H7 in meat formulations incorporating processed mustard powders (flours)

Food	Mustard flour processing treatment	% Mustard preparation in formulation	CFU/g reduction	No. days to achieve reduction
Dry cured Westphalian ham[16]	De-heated (cold) Yellow mustard flour	6%	5 $\log_{10}$	45
Ground beef under nitrogen-flushed packaging[15]	Regular (hot) Brown mustard flour	5%	0.5 $\log_{10}$	21 days
		10%	3.0 $\log_{10}$	21 days
		20%	5.1 $\log_{10}$	21 days
Dry fermented sausage[12]	De-heated (cold) mustard flour	6%	5 $\log_{10}$	38
(all Yellow mustard)	Regular (hot) mustard flour	6%	5 $\log_{10}$	31
	Autoclaved	6%	5 $\log_{10}$	18
Dry fermented sausage[8]	Regular (hot) Yellow mustard flour	2%	3.4 $\log_{10}$	30 d
		4%	4.4 $\log_{10}$	30 d
		6%	6.9 $\log_{10}$	30 d
Dry fermented sausage[8]	De-heated (cold) mustard flour	6%	Undetectable	24d

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Although AITC has been more extensively researched it has recently been found that because Yellow mustard has a greater glucosinolate content, it was a more potent antimicrobial agent than Oriental mustard. In addition, de-oiled mustard meal at 2% was as effective in eliminating live *E. coli* O157:H7 from dry fermented sausages during ripening as twice the concentration of mustard flour.[2,12] Deheated or deodorized (bland) mustard glucosinolates can be converted into highly antimicrobial isothiocyanate by bacterial myrosinase-like enzyme action present in *E. coli, 0157:H7*, *Staphylococcus carnosus* and *Pediococcus pentosaceus*[11,12,13] and in *L. monocytogenes*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Salmonella typhimurium*.[9]

Use in Antimicrobial Films

Incorporation of AITC or mustard flours into food formulations at too high a concentration (>4%) may adversely affect the sensory characteristics of foods. Thus, the incorporation of AITC or mustard extracts into antimicrobial films or coatings is an alternative way to capitalize on the antimicrobial properties of mustard and control food pathogens. Researchers are investigating ways to incorporate AITC into packaging such that the continuous release of minimal amounts will prolong the effective treatment time, enhance efficacy of AITC and reduce the impact of its odour. Research examples include:

- In edible coatings consisting of k-carrageenan/chitosan coatings, the addition of AITC, mustard extracts (from *B. jejuni*) alone or combined with EDTA, malic or acetic acid significantly reduced *C. jejuni* and *Salmonella* on fresh, refrigerated, vacuum-packed chicken breasts and *L. monocytogenes* on refrigerated, cured roast chicken[18,19]
- Incorporation of AITC into packaging labels inhibited the growth of fungi on cheese products[22]
- AITC was incorporated into high density polyethylene (HDPE) films and when combined with modified atmosphere packaging, inhibited the growth of *Salmonella typhimurium* and *Listeria monocytogenes* in fresh chicken samples[21]

Mustard Use Opportunities

- De-heated Yellow mustard flour could be used as an added natural antimicrobial to fermented meat to produce pathogen-free products
- Addition of AITC to edible packaging could be used to inhibit the growth of pathogens and spoilage organisms in processed meats, cheeses, nuts and fruits
- Incorporation of mustard essential oils in bakery products may be able to inhibit fungal growth and production of aflatoxins

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References

1. Cordeiro, R.P., Krause, D.O, Hernadez-Doria, J and Holley, R.A. 2014. *Food Microbiol*. 42:136-141
2. Cordeiro, R.P., Wu, C. and Holley, R.A. 2014. *Int. J. Food Microbiol*. 189:132-138
3. Davidson, P.M.D, Critzer, F.J. and Taylor, T.M. 2013. *Ann. Rev.Food Sci. Technol*. 4:163-190
4. Delaquis, P.J. and Scholberg, P.L. 1997*. J. Food Prot*. 60:943-947
5. Elabbasy, M.T., Eldesoky, K.I. and Morshdy, A.E. 2014. Life Sci. J. 11:185-190
6. Ekanayake, Vandiest, S.A., Kester, J.J., Zoutendam, P.H. and David, J.R.D. 2014 United States Patent US8,697,150
7. Ekanayake, A., Zoutendam, P.H.; Strife, R.J. Fu, X., and Jayatilake, G.S. 2012. *Food Chem*. 133:767-774
8. Graumann, G.H. and Holley, R.A. 2008. *J. Food Prot.* 71:486-493
9. Herzallah, S., Lledo, L., and Holley, R.a. 2011. *J. Food Prot.* 74:2162-2168
10. Lin, C.-M., Kim, J., Du, W.-X. And Wei, C. 2000. *J. Food Prot*. 63:727-734
11. Luciano, F., and Holley, R.A. 2009. *Int. J. Food Microbiol.* 131:240-245
12. Luciano, F.B. Belland, J. and Holley, R.A. 2011. *Int. J. Food Microbiol.* 145:69-76
13. Luciano, F.B. and Holley, R.A. 2010. *Fleischwirtsch Int.* 26:78-81
14. Monu, E.A., David, J.R.D, Schmidt, M and Davidson, P.M. 2014. *J. Food Prot.* 77:2062-2068
15. Nadarajah, D., Han, J.H. and Holley, R.A. 2005b. *Int. J. Food Microbiol.* 99:257-267
16. Nilson, A.M. and Holley, R.A. 2012. *Food Microbiol.* 30:400-407
17. Obaidat, M.M and Frank, J.F. 2009. *J. Food Prot.* 72: 6:315-324
18. Olaimat, A.N. and Holley, R.A. 2015. *Food Microbiol.* 48:83-88
19. Olaimat, A.N. and Holley, R.A. 2014. *J. Food Sci.* 79:M614-M621.57:90-95.
20. Schirmer, B.C. and Langrsrud, S. 2010. J. Food Sci. 75: M98-M102
21. Shin, J., Harte, B., Ryser, E and Selke, S. 2010. *J. Food Sci*: M65-M75
22. Winther, M., and Nielsen, P.V. 2006. *J. Food Prot.* 69:2430-2435

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