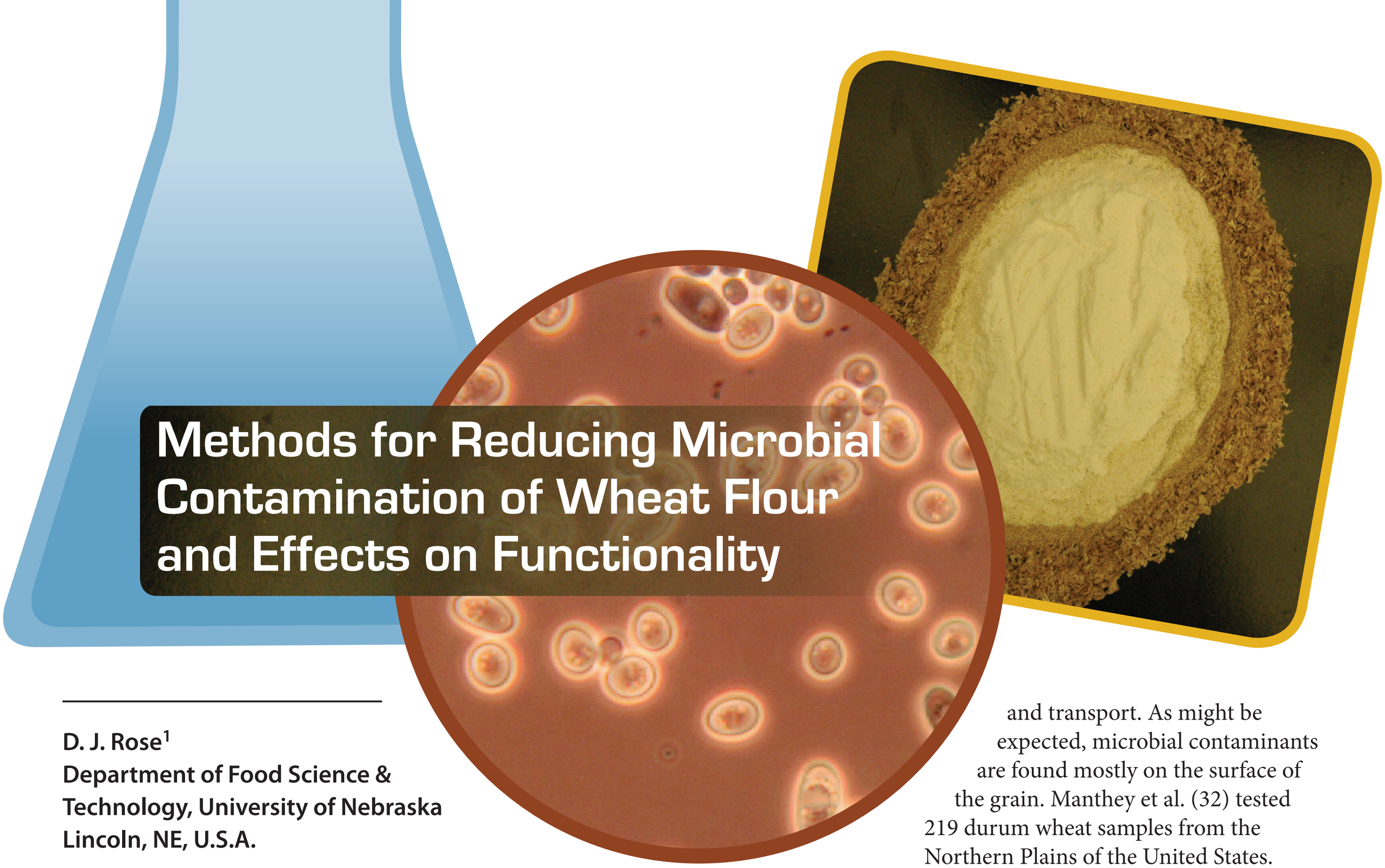

Methods for Reducing Microbial Contamination of Wheat Flour and Effects on Functionality

D. J. Rose[1]
Department of Food Science & Technology, University of Nebraska Lincoln, NE, U.S.A.

A. Bianchini, B. Martinez, and R. A. Flores
Department of Food Science & Technology, The Food Processing Center, University of Nebraska Lincoln, NE, U.S.A.

Wheat flour is a raw agricultural product and, thus, is subject to contamination from soil, animal feces, insects, diseased plants, and other agents (27). Historically contamination has been of little concern because it was assumed that the low water activity of wheat flour cannot support bacterial growth and that any pathogenic contaminant would be destroyed during baking, frying, or cooking of the flour used in final product processing. It has been discovered, however, that although wheat flour cannot support bacterial growth at normal storage moisture contents, bacteria can survive in a dormant state for extended periods in dry flour (12). Moreover, a recent national survey of 1,032 individuals in the United States indicated that 58% of consumers have tasted refrigerated cookie dough before baking and 80% have licked beaters after mixing batter for cakes, brownies, or muffins. Other products, including biscuit (24%), pie crust (22%), and pizza crust (11%) doughs are also occasionally consumed raw (7).

Based on these survey results, the assumption that wheat flour is microbiologically safe is risky for consumers. For example, in a recent widely publicized case 77 people in 30 states in the United States fell ill in May 2009 due to an *Escherichia coli* infection. While the specific strain responsible for the outbreak was not isolated from any food product, raw cookie dough was identified as the carrier, and the authors of the case report argued that it was the wheat flour that was contaminated (35).

Processing wheat flour to reduce microbial contamination presents challenges—not the least of which is that these treatments can reduce or affect flour functionality. The purpose of this article is to discuss processes aimed at reducing microorganisms in flour and their potential effects on flour functionality.

Wheat Flour Microbiology

As a raw agricultural product, wheat carries with it contaminants from the environments it is exposed to during production, including harvest, storage, and transport. As might be expected, microbial contaminants are found mostly on the surface of the grain. Manthey et al. (32) tested 219 durum wheat samples from the Northern Plains of the United States. They found that wheat as received from growers, farm bins, and elevators contained 1.6×10^8 cfu of bacteria per gram. After cleaning with a dockage tester and cyclone grain cleaner the microbial load was reduced by 1 log. Milling the cleaned wheat using a Buhler experimental mill further reduced the microbial count to 5.0×10^5 cfu/g in the straight-grade flour. Laca et al. (27) removed 1.4–22% of the outer portions of wheat kernels using an abrasive test mill and analyzed the remaining grain for microbial load. They found a 1 log reduction in bacteria after removing only the outermost 4% of the grain.

Although the milling process removes much of the outer layers of the wheat kernel, flour can still retain unsafe contaminants. Several studies have determined the microbial load in a large number of wheat flour samples (Table I). Total bacteria, mold, and yeast contents were fairly consistent among flour samples analyzed, but wide discrepancies existed with respect to specific pathogens. For example, the causative agent in the abovementioned food-borne illness outbreak (35), *E. coli*, was identified at >9 cfu/g in a staggering 72 of 142 samples analyzed in Turkey (2), while only 2 of 300 samples were positive for *E. coli* in a study conducted in Australia (12). In this same set of samples from Australia, *Bacillus cereus* was not detected in any

[1] Corresponding author. Department of Food Science & Technology, University of Nebraska-Lincoln, 252 Food Industry Complex, Lincoln, NE 68583, U.S.A. E-mail: drose3@unl.edu; Tel: +1.402.472.2802.

http://dx.doi.org/10.1094/CFW-57-3-0104

samples (12), while 93% of samples from another study in Australia were positive for *B. cereus* (4). *Salmonella* sp. was detected in a few samples from the United States (39) (Table I).

Although contamination with pathogenic bacteria may seem low based on the data presented in Table I, these bacteria can cause illness at very low doses. Moreover, with the wide distribution of food commodities that is common in today's marketplace, a single contaminated batch of flour can result in widespread illness and a tarnished company image.

Strategies to Reduce Microbial Count Prior to Milling

When wheat is received at the mill, it contains chaff, stones, and other foreign materials that must be removed before milling (37). Wheat kernels are cleaned using screens, air currents, brushes, and magnets prior to tempering (37). Although these steps reduce the total microbial load by ≈1 log, the wheat kernels still contain a substantial number of contaminants (32), and there are no reports on the effectiveness of these cleaning steps for pathogen reduction. Several authors have explored new cleaning steps to further reduce total bacterial populations.

Ozone is a powerful oxidizing agent that is used in the food industry as a disinfectant. It is effective against insects (23) and pathogenic and spoilage microorganisms (38) and has been shown to degrade mycotoxins (52). One of the more attractive features of ozone is that it leaves no chemical residue because it degrades to oxygen gas. With respect to wheat, ozone has been most extensively studied as a fumigant applied during bulk storage of wheat grain to reduce pest contamination (8,19,51).

Ibanoglu (22) washed wheat with ozonated water (1.5 mg/L). In the study, 500 g of wheat was washed with 1 L of ozonated water for 30 min, drained, and dried in a forced-draft oven at 30°C to 16.1 and 17% moisture for soft and hard wheats, respectively, before experimental milling. This treatment resulted in an ≈1 log reduction in total bacteria, yeast, and mold compared with a similar water wash.

Dhillon et al. (11) showed that treatment with ozonated (16.5 mg/L) or chlorinated (700 mg/L) water at 2 L/kg of durum wheat for 3 min was effective at reducing yeast and mold counts by 0.5 and 2 logs, respectively, although neither treatment affected bacterial load. The lack of an effect on bacteria in this study may have been due to the short contact time with ozone compared with the previous study. Acidic pH (1% acetic acid) increased dissolved ozone in the wash water and increased ozone half-life. Therefore, Dhillon et al. (11) also treated wheat with ozonated water (20.5 mg/L) in 1% acetic acid at 2 L/kg of wheat for 3 min. This was remarkably effective at reducing microbial contamination: the aerobic plate count was reduced from 7.9×10^4 cfu/g to 50 cfu/g, and the yeast and mold count was reduced from 1.9×10^4 cfu/g to <10 cfu/g. In a follow-up study, Dhillon et al. (10) created a fluidized bed of wheat using air currents and applied gaseous ozone or ozone dissolved in water or acetic acid solution. A combination of gaseous ozone (6 ppm), acetic acid (0.5%), and ozonated water (26 mg/L) was most effective at reducing microbial load: 1.7 log for bacteria and 3.3 log for mold and yeast.

Although ozone is a powerful oxidant, Tiwari et al. (44) noted that oxidation by ozone is mostly confined to the outer layers of the grain. Thus, ozonation of whole-kernel wheat is likely to have a minimal effect on the functional properties of flour, which comes from the center of the grain. Indeed, in rice treated with ozone, extensive damage was observed in the intact grain after treatment, including a musty odor and darker color; however, after the bran was removed the differences were no longer evident (33). Ibanoglu (22) studied the effects of washing wheat kernels with ozonated water (1.5 mg/L at 2 L/kg of wheat for 30 min) on flour extraction rate, farinograph and extensograph characteristics, protein, falling number, and sedimentation volume of the resulting flour. Ozone treatment did not affect any of these properties except dough extensibility, for which there was a small, albeit significant, increase.

Desvignes et al. (8) exposed wheat to a much more extensive ozone treatment that lasted up to 5 hr. They found that ozone treatment reduced the energy requirement at the break stage of milling and reduced coarse bran yield. Biochemical characterization of the flour indicated a reduction in starch damage, an increase in aleurone in the flour, and an increase in insoluble glutenin polymers. These changes in flour properties might be attributable to increased oxidation as a result of excessive ozonation.

Although fewer reports are available, strategies other than ozonation may be used to reduce microbial contamination. For instance, in the study by Dhillon et al. (11) in which they examined acidified (acetic acid) ozone treatment of wheat kernels, the 1% acetic acid solution without addition of ozone was equally effective at reducing bacteria and almost as effective at reducing yeast and mold compared to the solution with added ozone. Vojnovich et al. (46) studied the effectiveness of washing corn with hot water (64–100°C) or steam alone or in combination with a variety of chlorine-based sanitizing agents. Submersing corn in water at 82°C for 60 min or steam for 30 min before milling reduced bacteria in the flour (hammer milled) from 1×10^6 to 7×10^2 cfu/g. Corn treated with chlorine-based sanitizers dissolved in water at 65°C for 1–3 min reduced the bacterial load by 1–3 logs depending on treatment. The effectiveness and appropriateness of these strategies for reducing microbial load in wheat and their effects on the functional properties of flour have not been explored.

A few investigators have studied the value of adding antimicrobial agents to tempering water as a means of reducing bacterial contamination. Chlorinated water (600–700 ppm) can be added to tempering water to reduce the bacterial load (9). Chlorination, however, leaves potentially hazardous residues;

Table I. Microorganisms in wheat flour

	Origin[a]			
Category	Turkey (ref. 2)	Australia (ref. 4)	Australia (ref. 12)	United States (ref. 39)
Bacteria (log cfu/g)	4.14 (142)	NR	4.2 (100)	4.17 (1,354)
Mold (log cfu/g)	2.24 (142)	NR	2.80 (50)	2.90 (1,682)
Yeast (log cfu/g)	NR	NR	3.70 (50)	2.12 (1,648)
Escherichia coli (% positive)	51 (142)	1 (71)	2 (300)	12.8 (3,350)
Salmonella sp. (% positive)	ND (142)	NR	ND (150)	1.3 (3,040)
Bacillus cereus (% positive)	4.2 (142)	93 (71)	ND (350)	NR

[a] Values are means; sample sizes are provided in parentheses. NR = Not reported; ND = not detected.

therefore, Dhillon et al. (9) tempered durum and hard red spring wheats with water containing ozone at 16 mg/L. Ozone treatment did not appreciably affect microbial load (<1 log difference) compared with traditional tempering.

In a similar experiment, Ibanoglu (21) tempered hard red and soft white wheats to 16.5 and 15.5% moisture, respectively, with water containing ozone (11.5 mg/L). In hard wheat, bacteria, yeast, and mold were reduced by ≈1 log. In soft wheat, bacteria was reduced by ≈2 logs, and yeast and mold were reduced by 1 log. Minor differences in ozone application or other methodologies between this and the previous study might account for the different outcomes. Ibanoglu (21) also measured milling extraction rate, farinograph and extensograph characteristics, protein, falling number, sedimentation volume, and color for ozone-tempered wheat. No changes were observed in any of these analyses compared with the control.

Strategies to Reduce Microbial Counts After Milling

Heat. Dry heating of wheat flour, especially substandard wheat flour, has long been recognized as a means of improving functional properties such as water absorption, farinograph quality number, gluten strength, adhesiveness, and color (6,14–16,25,34,36,40,42,43,45,50). The effects of heat on wheat flour, when applied appropriately, are similar to chemical bleaching. Appropriate treatments vary widely: 80–180°C for 5 sec to 15 min. This is perhaps due to differences among wheat classes but more likely is due to differences in flour moisture contents and heating methods. Indeed, heating for too long (e.g., 170°C for 15 min [49]) can result in browning of the flour, while too much moisture (e.g., 80°C for 30 min at 20% moisture [48]) can result in gluten denaturation.

Because heat treatments are often used to reduce or eliminate microorganisms in foods, the possibility of using heat treatments to reduce contaminants in flour has been reported in a few studies. Upreti et al. (45), for instance, heated flour that had been evenly dispersed on a baking sheet (0.1 cm thickness) at 290°C for 5 min in a hot-air oven. Total bacteria was reduced from 2,700 cfu/g before treatment to120 cfu/g after treatment. Wiseblatt et al. (49) heated flour (6.5 mm depth) that had been inoculated with *Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus flavus*, or *E. coli* in a hot-air oven at 130°C for 45 min. This treatment was remarkably effective at reducing the viability of these organisms: *B. cereus* was reduced by 2 logs, *Staphylococcus aureus* was reduced by 4 logs, and *A. flavus* and *E. coli*, inoculated at 710 and 300 cfu/g, were not detected after treatment.

As stated previously, moisture content is very important with respect to the effect of heat treatment on flour functionality. Laroche et al. (28) demonstrated this using flour samples that were spiked with *Saccharomyces cerevisiae* or *Lactobacillus plantarum*. They found that the viability of the microorganisms after treatment was reduced when the initial water activity of the flour was between 0.3 and 0.5. However, when water activity was very low (0.1) or high (>0.5) the spiked organisms were effectively inactivated. Although this implies that very dry flour might be treated to effectively inactivate microorganisms, Upreti et al. (45) showed that heating flour with <1.5% moisture content reduced functionality. Thus, heating flour with sufficient moisture to effectively inactivate microorganisms while not denaturing gluten or gelatinizing starch is a constant challenge.

More elaborate heat treatments than simply heating flour in an oven have been developed to overcome some of these challenges. In one new technology, a dry product is dehydrated at low temperature (70–90°C) and then treated with steam to inactivate microorganisms. The increased moisture generated by the steam is then removed under vacuum (30). In another approach, the dry product is fed into a chamber with a rotating, heated screw and dry steam injection. As the product is propelled through the chamber by the rotating screw, moisture lost from the product is removed by air circulation (www.safesteril.com). These technologies have been used mainly for pasteurization of spices and nuts but may also be applicable to flour.

Irradiation. Gamma rays are a type of ionizing radiation that has been approved for inactivating microorganisms in some foods. Irradiation is remarkably effective at reducing pathogen and total microbial counts but may cause changes to the food product, such as darkening of meat (5) or destruction of antioxidants (1). In wheat, irradiation has most often been used to kill insects during bulk storage of wheat kernels. Only a few studies have reported on irradiation of wheat flour. For instance, Hanis et al. (18) treated whole milled wheat with 1, 10, and 25 kGy of radiation from a ^{60}Co source. In the study, 1 kGy reduced viable microorganisms by 2 logs, while 10 kGy completely eliminated bacteria. Zaied et al. (53) treated flour with 2, 4, and 8 kGy of gamma radiation. They found a substantial increase in reducing sugars and decrease in dough development time and gluten strength in flours treated with 4 and 8 kGy of gamma radiation. In a sensory panel, participants ranked these samples as less acceptable than the control and the sample made from flour treated with 2 kGy of radiation. Koksel et al. (26) also reported decreases in dough quality parameters in flour treated with high doses of radiation (>2.5 kGy).

Other forms of radiation, such as ultraviolet (UV), have been used to inactivate microorganisms in foods. Laszlo et al. (29) used UV radiation to reduce microorganisms in wheat flour, but the treatment did not affect the microbial load. When UV radiation was combined with ozone (20 mg/kg of flour), microbial load was reduced by ≈2 logs, and it is probable that the reduction obtained in the combined treatment was caused by the ozone.

Microwave and radio frequency irradiation can also be used to reduce microbial contaminants in foods. These types of non-ionizing radiation mainly affect microorganisms through generation of heat. An advantage of using this mode of heating is that it is rapid compared with conventional heating methods. Unfortunately, heating is uneven, and steps to ensure more even heating must be taken (20).

A new treatment process has been described that uses radio frequency irradiation to inactivate microorganisms in wheat flour (47). Flour was treated with a radio frequency device to increase the temperature to 75–100°C followed by cooling with cold air. Depending on the severity of the treatment, a 4–7 log reduction in bacteria was reported. The authors claim this treatment resulted in minimal effects on flour functionality.

There are no reports on the destruction of microorganisms in flour using microwave radiation. When exploring the effects of microwaves on the functional properties of flour, MacArthur and D'Appolonia (31) treated 2 kg of flour at ≈14% moisture in a household microwave oven (625 W) for periods of up to 6 min. They noted a substantial loss in baking quality when the flour was heated for more than 4 min. At 4 min, the temperature of the flour was 61°C; thus, minimal microorganism destruction would be expected. A higher powered microwave or flours with different moisture contents might result in an effective treatment for reducing microorganism viability without affecting the functional properties of flour.

Ozone. Ozone may be used to inactivate microorganisms in wheat flour; however, because the flour is not protected from oxidation by the pericarp, as when treating whole-kernel wheat, ozone readily oxidizes flour components. When applied at moderate doses, ozone can act similarly to other oxidizing agents such as potassium bromate (41). Sandhu et al. (41) packed wheat flour in a cylinder through which ozone gas was pumped. They found that after 45 min of treatment with ozone at 1,500 mg/kg, the flour was fully oxidized (no further ozone consumption) and produced unacceptable bread. However, if flour was treated for only 4.5 min or if fully oxidized flour was mixed with untreated flour at 1:9 (wt/wt), respectively, bread appearance and volume were improved. The effect of this treatment on microbial load was not reported.

Other. As is evidenced in this article, there is a lack of data showing inactivation of pathogens in wheat or flour as a result of specific treatments. This is because, as was mentioned previously, the issue of pathogens in wheat flour is a new concern for the baking industry. Therefore, there remain many unexplored treatments and technologies that may prove effective at reducing pathogens in flour. Included among these technologies are pulsed electric field (24) and high-pressure processing (13), although these treatments may not be effective without added moisture. Ensuring the safety of wheat flour without affecting its functional properties may require a combination of treatments in a hurdle-like approach.

Post-treatment Considerations

Most wheat flour mills and supply chains are not designed to deliver a ready-to-eat product. Postprocessing contamination can occur during milling if the treatment to reduce microbial load is applied to the whole wheat kernels or during packaging, shipping, or receiving if the treatment is applied to the flour. Therefore, prevention of microbial establishment and growth within the mill and throughout the supply chain is an integral part of the control measures used to reduce the microbial load in flour.

In the prevention of microbial establishment and growth within the mill, the roles played by sanitary facilities and sanitary equipment design are important. The facilities should 1) be of adequate size to produce the volume of product required; 2) have an adequate storage capacity for the finished product and ingredients in the event that a load of ingredients needs to be rejected; 3) allow for adequate cleaning and sanitization; and 4) have an adequate potable water source with pressure and temperature control. The equipment should have a sanitary design, including construction with materials that are compatible with wheat and flour, are easy to clean, are accessible, do not have hollow areas or niches, provide dust extraction capability, and have sanitary operational performance (17).

In a dry environment such as a milling facility, it is also critical to avoid any potential introduction of water or moisture, because it is a main contributing factor to the growth and establishment of microorganisms. If microbial inactivation is obtained by thermal treatment, the product should be cooled and, if needed, dried immediately after processing to avoid formation of condensation in buildings and equipment. After processing, it is also important to package the product aseptically and in containers that will minimize any form of condensation.

It is also important that current Good Manufacturing Practices be followed throughout the facility (described in the *Code of Federal Regulations*, title 21, part 110). If an inactivation step is introduced as part of the milling process, segregation into a basic-hygiene area and a high-hygiene area could be incorporated as part of the effort to minimize cross-contamination between untreated and final products. The high-hygiene area is defined as the area where handling of ingredients and products requires the highest level of hygiene control and is subsequent to the lethality step (17). Employees working in this area should be properly trained so they understand their critical role in preventing cross and postprocessing contamination. The use of positive air pressure is generally recommended, as well as filtered air for cooling. Traffic and processing flow patterns should be designed to avoid contact between the finished product and raw materials, and dedicated equipment, utensils, and tools should be used in basic- and high-hygiene areas.

In addition to the measures discussed here, an environmental monitoring program (EMP) can also be used to verify the effectiveness of the control program on an ongoing basis through evaluation of the production environment, its cleanliness, and the microbial load present in the facility and on equipment. Data generated from an EMP can be used for trend analysis to identify harborage sites for microorganisms and assess the effectiveness of cleaning and sanitizing programs.

The concept of zone sampling, introduced by Kraft Foods, is widely used for determining sampling sites in an EMP (3). The concept divides plant operations into four zones based on level of risk: 1) product contact surfaces (e.g., conveyors, filter hoppers, scrapers, packaging equipment, and employee hands); 2) non-product contact surfaces (e.g., equipment exterior, catwalks, control panels, HVAC vents, and weight scales); 3) other non-product contact surfaces within the high-hygiene area (e.g., cleaning tools, transition rooms, floor drains, and wash stations); and 4) non-product surfaces outside the high-hygiene area (e.g., raw materials area, employee break room, storage rooms, and laboratories). Indicator organisms are usually monitored in zone 1. Pathogen monitoring is performed only under special circumstances in this zone, while zones 2–4 are monitored for the pathogen of interest. Analyses may include nonpathogenic *E. coli*, Enterobacteriaceae, yeast and mold, and pathogenic organisms. Positive results or trend data above the established baseline require corrective actions. Thus, as part of the EMP, measures should be established to contain potential contamination, identify potential sources of contamination, assess the potential for finished product contamination, and eliminate problems. Problem areas should be retested with increased frequency after corrective actions are taken until negative results or baseline results are achieved.

A well-established EMP can be a very good measuring tool to evaluate how effective efforts applied during all phases of processing are at reducing or maintaining the microbial load in the final product. It is also more practical and reliable to monitor the processing environment than to rely solely on testing of the finished product.

Conclusions

Recent food-borne illness outbreaks have created new concerns about the safety of wheat flour and a number of treatments, including heat, ozone, and irradiation, for wheat grain and flour have been proposed as strategies to improve the microbiological safety of this product. These treatments may be effective; however, literature documenting pathogen destruction in wheat flour as a result of these and other treatments is lacking. It is clear that regardless of the treatment applied, processing conditions need to be tightly controlled, because flour components that are important for functionality, including gluten and starch, can be easily modified as a result of these treatments. New sanitation techniques, packaging, and shipping strategies may be needed, in addition to the processing methods and control measures discussed here, before wheat flour can be considered a ready-to-eat product.

References

1. Alothman, M., Bhat, R., and Karim, A. A. Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends Food Sci. Technol. 20:201, 2009.
2. Aydin, A., Paulsen, P., and Smulders, J. M. The physico-chemical and microbiological properties of wheat flour in Thrace. Turk. J. Agric. For. 33:445, 2009.
3. Baldus, K., and Deibel, V. Sanitation—How to validate your GMP and prerequisite programs. Food Safety Mag. April/May, 2007.
4. Berghofer, L. K., Hocking, A. D., Miskelly, D., and Jansson, E. Microbiology of wheat and flour milling in Australia. Int. J. Food Microbiol. 85:137, 2003.
5. Brewer, S. Irradiation effects on meat color—A review. Meat Sci. 68:1, 2004.
6. Chigurupati, S. R., and Pulverenti, J. Method for increasing stability and bake absorption of a bread baking wheat flour and resulting dough. U.S. patent 5,352,473, 1994.
7. ConAgra Mills. The raw truth about consumer eating habits. Published online at www.conagramills.com/media/Food%20Habits%20of%20American%20Consumers%20Final.pdf. ConAgra Mills Consumer Insights, Omaha, NE, 2011.
8. Desvignes, C., Chaurand, M., Dubois, M., Sadoudi, A., Abecassis, J., and Lullien-Pellerin, V. Changes in common wheat grain milling behavior and tissue mechanical properties following ozone treatment. J. Cereal Sci. 47:245, 2008.
9. Dhillon, B., Sandhu, H., Wiesenborn, D., Manthey, F., and Wolf-Hall, C. A comparison between chlorinated water and ozonated water as an antimicrobial treatment during tempering of wheat. Published online at http://asae.frymulti.com/azdez.asp?JID=5&AID=23345&CID=min2007&v=&i=&T=1&refer=7&access=. American Society of Agricultural and Biological Engineers, St. Joseph, MI, 2007.
10. Dhillon, B., Wiesenborn, D., Dhillon, H., and Wolf-Hall, C. Development and evaluation of a fluidized bed system for wheat grain disinfection. J. Food Sci. 75:E372, 2010.
11. Dhillon, B., Wiesenborn, D., Wolf-Hall, C., and Manthey, F. Development and evaluation of an ozonated water system for antimicrobial treatment of durum wheat. J. Food Sci. 74:E396, 2009.
12. Eglezos, S. Microbiological quality of wheat grain and flour from two mills in Queensland, Australia. J. Food Prot. 73:1533, 2010.
13. Estrada-Girón, Y., Swanson, B. G., and Barbosa-Cánovas, G. V. Advances in the use of high hydrostatic pressure for processing cereal grains and legumes. Trends Food Sci. Technol. 16:194, 2005.
14. Geddes, W. F. Chemical and physico-chemical changes induced in wheat and wheat products by elevated temperatures–I. Can. J. Res. 1:528, 1929.
15. Geddes, W. F. Chemical and physico-chemical changes induced in wheat and wheat products by elevated temperatures–II. Can. J. Res. 2:65, 1930.
16. Gelinas, P., McKinnon, C. M., Rodrigue, N., and Montpetit, D. Heating conditions and bread-making potential of substandard flour. J. Food Sci. 66:627, 2001.
17. Grocery Manufacturers Association. Control of *Salmonella* in low-moisture foods. Published online at www.gmaonline.org/downloads/technical-guidance-and-tools/SalmonellaControlGuidance.pdf. GMA, Washington, DC, 2009.
18. Hanis, T., Mnukova, J., Jelen, P., Klir, P., Perez, B., and Pesek, M. Effect of gamma irradiation on survival of natural microflora and some nutrients in cereal meals. Cereal Chem. 65:381, 1988.
19. Hardin, J. A., Jones, C. L., Bonjour, E. L., Noyes, R. T., Beeby, R. L., Eltiste, D. A., and Decker, S. Ozone fumigation of stored grain; Closed-loop recirculation and the rate of ozone consumption. J. Stored Prod. Res. 46:149, 2010.
20. Hui, Y. H., Lim, M. H., Nip, W., Scott, J., and Yu, P. Principles of food processing. Pages 3-25 in: *Food Processing Principles and Applications*. J. Scott and Y. Hiu, eds. Blackwell Press, Ames, IA, 2004.
21. Ibanoglu, S. Influence of tempering with ozonated water on the selected properties of wheat flour. J. Food Eng. 48:345, 2001.
22. Ibanoglu, S. Wheat washing with ozonated water: Effects on selected flour properties. Int. J. Food Sci. Technol. 37:579, 2002.
23. Isikber, A. A., and Oztekin, S. Comparison of susceptibility of two stored-product insects, *Ephestia kuehniella* Zeller and *Tribolium confusum* du Val, to gaseous ozone. J. Stored Prod. Res. 45:159, 2009.
24. Keith, W. D., Harris, L., and Griffiths, M. W. Reduction of bacterial levels in flour by pulsed electric fields. J. Food Process Eng. 21:263, 1998.
25. Kent-Jones, D. W. Some aspects of the effect of heat upon flour. Cereal Chem. 5:235, 1928.
26. Koksel, H., Sapirstein, H. D., Celik, S., and Bushuk, W. Effects of gamma-irradiation of wheat on gluten proteins. J. Cereal Sci. 28:243, 1998.
27. Laca, A., Mousia, Z., Diaz, M., Webb, C., and Pandiella, S. S. Distribution of microbial contamination within cereal grains. J. Food Eng. 72:332, 2006.
28. Laroche, A., Fine, F., and Gervais, P. Water activity affects heat resistance of microorganisms in food powders. Int. J. Food Microbiol. 97:307, 2005.
29. László, Z., Hovorka-Horváth, Z., Beszédes, S., Kertész, S., Gyimes, E., and Hodúr, C. Comparison of the effects of ozone, UV, and combined ozone/UV treatment on the colour and microbial counts of wheat flour. Ozone Sci. Eng. 30:413, 2008.
30. Log5. CCP pasteurization and sterilization of dry foods. Published online at www.log5.com/PDF/Log5_CCP_Brochure_Print.pdf. Log5 Corporation, Phoenix, MD, 2010.
31. MacArthur, L. A., and D'Appolonia, B. L. Effects of microwave radiation and storage on hard red spring wheat flour. Cereal Chem. 58:53, 1981.
32. Manthey, F. A., Wolf-Hall, C. E., Yalla, S., Vijayakumar, C., and Carlson, D. Microbial loads, mycotoxins, and quality of durum wheat from the 2001 harvest of the Northern Plains region of the United States. J. Food Prot. 67:772, 2004.
33. Mendez, F., Maier, D. E., Mason, L. J., and Woloshuk, C. P. Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. J. Stored Prod. Res. 39:33, 2003.
34. Nakamura, C., Koshikawa, Y., and Seguchi, M. Increased volume of *kasutera* cake (Japanese sponge cake) by dry heating of wheat flour. Food Sci. Technol. Res. 14:431, 2008.
35. Neil, K. P., Biggerstaff, G., MacDonald, J. K., Trees, E., Medus, C., Musser, K. A., Stroika, S. G., Zink, D., and Sotir, M. J. A novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: Multistate outbreak of *E. coli* O157:H7 infections associated with consumption of

ready-to-bake commercial prepackaged cookie dough—United States, 2009. Clin. Infect. Dis. (online) doi:10.1093/cid/cir831, 2012.
36. Ozawa, M., and Seguchi, M. Relationship between pancake springiness and interaction of wheat flour components caused by dry heating. Food Sci. Technol. Res. 12:167, 2006.
37. Posner, E. S., and Hibbs, A. N. *Wheat Flour Milling*. AACC International, St. Paul, MN, 1997.
38. Restaino, L., Frampton, E., Hemphill, J., and Palnikar, P. Efficacy of ozonated water against various food related micro-organisms. Appl. Environ. Microbiol. 61:3471, 1995.
39. Richter, K. S., Dorneanu, E., Eskridge, K. M., and Rao, C. S. Microbiological quality of flours. Cereal Foods World 38:367, 1993.
40. Russo, J. V., and Doe, C. A. Heat treatment of flour as an alternative to chlorination. Food Technol. 5:363, 1970.
41. Sandhu, H. P. S., Manthey, F. A., and Simsek, S. Quality of bread made from ozonated wheat (*Triticum aestivum* L.) flour. J. Sci. Food Agric. 91:1576, 2011.
42. Seguchi, M. Effect of heat-treatment of wheat flour on pancake springiness. J. Food Sci. 55:784, 1990.
43. Thomasson, C. A., Miller, R. A., and Hoseney, R. C. Replacement of chlorine treatment for cake flour. Cereal Chem. 72:616, 1995.
44. Tiwari, B. K., Brennan, C. S., Curran, T., Gallagher, E., Cullen, P. J., and O'Donnell, C. P. Application of ozone in grain processing. J. Cereal Sci. 51:248, 2010.
45. Upreti, P., Roberts, J. S., and Jalali, R. Heat-treated flour. U.S. patent application 20100092639, 2010.
46. Vojnovich, C., Pfiefer, V. F., and Griffin, E. L. Reducing microbial populations in dry-milled corn products. Cereal Sci. Today 15:401, 1970.
47. Weaver, G., Akins-Lewenthal, E., Allen, B., Baker, S., Hoerning, D., Peterson, A., Schumacher, R., and Warren, B. Microbial reduction in a processing stream of a milled product. U.S. patent application 20110177216, 2011.
48. Weegels, P. L., Verhoek, J. A., de Groot, A. M. G., and Hamer, R. J. Effects on gluten of heating at different moisture contents. I. Changes in functional properties. J. Cereal Sci. 19:31, 1994.
49. Wiseblatt, L. Reduction of the microbial populations in flour incorporated into refrigerated foods. Cereal Chem. 44:269, 1967.
50. Wolt, M., Chigurupati, S. R., and Pulverenti, J. Method for heat treating a bread baking wheat flour and resulting flour and dough. U.S. patent 5,433,966, 1995.
51. Wu, J. N., Doan, H., and Cuenca, M. A. Investigation of gaseous ozone as an antifungal fumigant for stored wheat. J. Chem. Technol. Biotechnol. 81:1288, 2006.
52. Young, J. C. Reduction in levels of deoxynivalenol in contaminated corn by chemical and physical treatment. J. Agric. Food Chem. 34:465, 1986.
53. Zaied, S. E. A. F., Abdel-Hamid, A. A., and Attia, E. S. A. Technological and chemical characters of bread prepared from irradiated wheat flour. Nahrung 40:28, 1996.

Devin Rose received his Ph.D. degree from Purdue University; his with research focused on creating slowly fermentable dietary fibers for improving gut health. After completing his Ph.D. degree, he worked as a post-doc with the USDA Agriculture Agricultural Research Service on creating healthy, functional food ingredients from by-products of the grain milling industries. Devin is currently an assistant professor at the University of Nebraska-Lincoln with research interests in whole-grain processing and gut health. Devin is an AACC International member and can be reached at drose3@unl.edu.

Andréia Bianchini is a research assistant professor in The Food Processing Center at the University of Nebraska-Lincoln. She has a B.S. degree in food engineering and an M.S. degree in environmental and agricultural microbiology, both from Brazil. Her Ph.D. degree is in food science and technology from the University of Nebraska-Lincoln. With a strong background in food safety and molds and mycotoxins, her area of interest includes the development of quality control mechanisms and HACCP assistance, focusing on food, dairy, and feed products. Andréia conducts applied research and provides technical advice and training activities for improvement of the safety and quality of food and pet food processes. She is a member of the International Association for Food Protection and the Institute of Food Technologists. Andréia is an AACC International member and can be reached at abianchini2@unl.edu.

Bismarck Martinez is from Nicaragua. He received his B.S. degree in food science and technology from Zamorano University in Honduras. Currently, he is a graduate student in the Department of Food Science & Technology at the University of Nebraska-Lincoln. He is working for the Food Processing Center in the microbiological analysis laboratory. His research is on *E. coli* O157:H7 contamination of field crops. Bismarck can be reached at bamt3682@hotmail.com.

Rolando A. Flores is professor and head of the Food Science and Technology Department and director of The Food Processing Center at the University of Nebraska-Lincoln. He holds a B.S. degree in mechanical engineering from the Universidad de Costa Rica; an M.S. degree in agricultural engineering from Iowa State University (ISU); and a Ph.D. degree in grain science and industry from Kansas State University (KSU). Rolando began his career working with the production, storage, and transportation of agricultural products at the National Production Bureau in Costa Rica. He has held faculty positions at KSU and ISU and research positions at the Eastern Regional Research Center of the USDA Agricultural Research Service. He has conducted research on value-added agricultural products, particle reduction and fractionation of grain products, modeling and simulation of food processing systems, food safety risk process analysis, and optimization of coproduct utilization of ethanol from corn and barley. He has published more than 80 articles in refereed journals and given more than 130 presentations at scholarly conferences. Rolando has received several professional awards, such as the AACC International Engineering and Processing Division Stanley Watson Award and the USDA–ARS 2010 Technology Transfer Award. Rolando is an AACC International member and can be reached at rflores2@unl.edu.