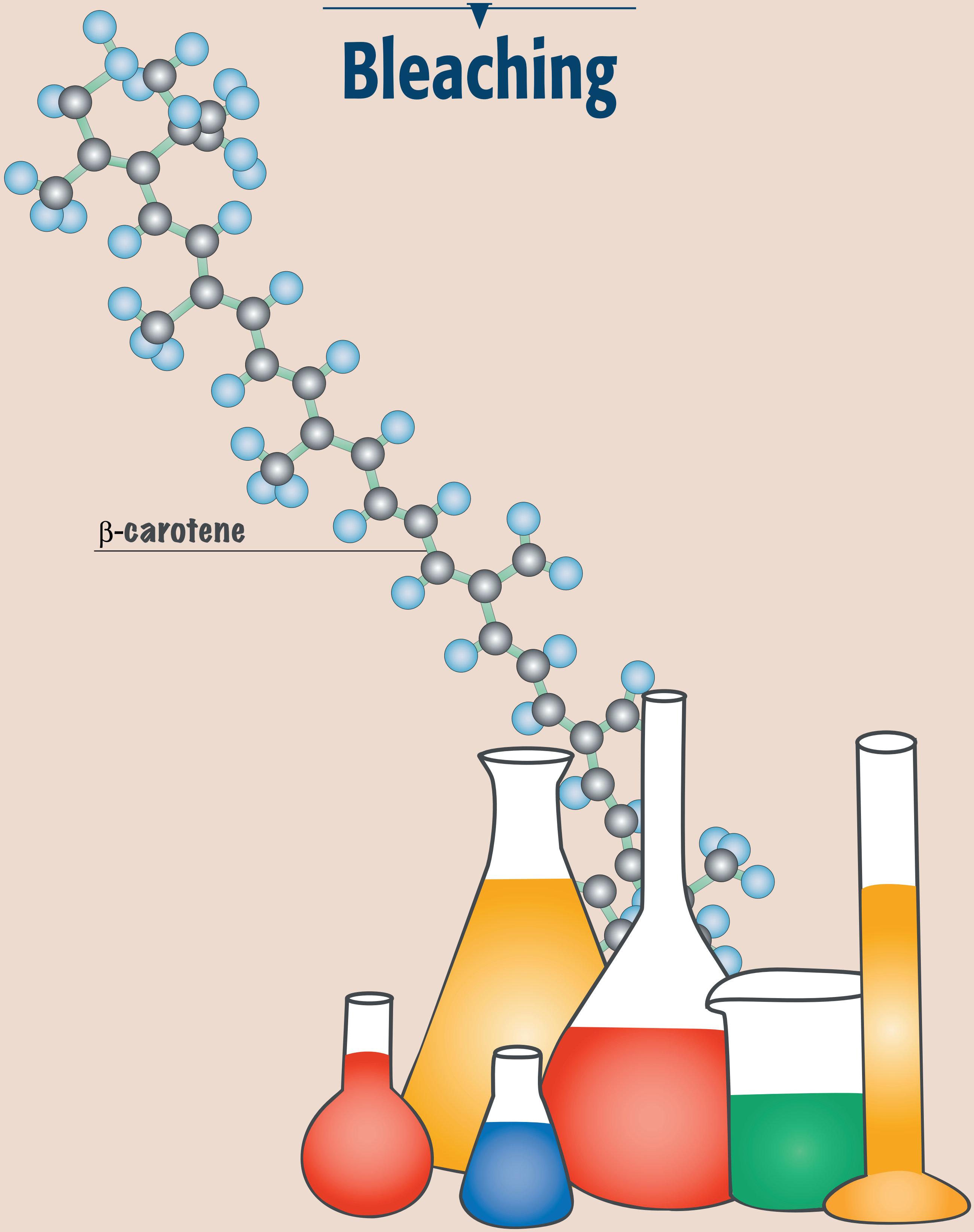
Whey Processing CDR Technical Review

Bleaching



Wisconsin Center for Dairy Research

Whey Processing CDR Technical Review

Bleaching

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This document, prepared by the Wisconsin Center for Dairy Research and funded by Dairy Management, Inc, is intended to help clarify the effects of whey production and handling on whey flavor. The document includes commonly used dairy terms, manufacturing and processing technology, and applicable technical information. When citing this information as a reference, please credit the Wisconsin Center for Dairy Research, the document name and date of the publication.

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Introduction

Whey may be bleached to lighten color compounds that have been added to cheesemilk. Benzoyl and hydrogen peroxide are legally permitted bleaching agents. Hydrogen peroxide also may be used to limit growth of bacteria during electrodialysis of whey. Although widely practiced, peroxide may not be used to control acid production by bacteria during storage of whey or as a substitute for Good Manufacturing Practices (GMPs).

Sources of Color in Whey

There are three general sources of color in whey: xanthophylls; Maillard reaction products and; annatto.[2] The ability of peroxide to remove color from the whey depends on the color compound present.

Xanthophylls belong in the class of pigments referred to as carotenoids. Carotenoids are tetraterpenes. Found in fruits and vegetables, carotenes range in color from yellow to orange, red and brown. More than 600 carotenoids occur in nature and their color largely is a function of the number of conjugated double bonds in the molecule. Xanthophylls in the form of β-carotene (Figure 1) enter milk through forage eaten by cows. Incomplete conversion of β-carotene to vitamin A in the mammary gland results in a yellow color in the milk. Hydrogen and benzoyl peroxide will bleach xanthophylls, however, regulations regarding bleaching xanthophylls in milk do not permit the use of hydrogen peroxide.

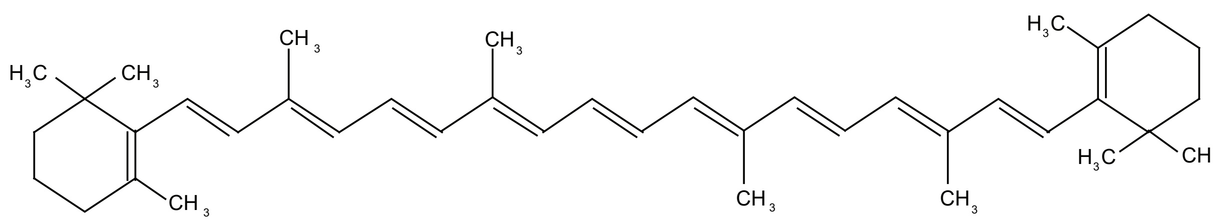


Figure 1. Structure of carotene

Maillard reaction products range in color from tan to dark brown and result from the interactions between amino compounds and sugars. Neither hydrogen nor benzoyl peroxide will remove color caused by the Maillard reaction.[2]

Annatto is the yellow/orange pigment used to color cheese. Annatto is an extract of the pericap or fruit wall of the shrub Bixa orellana and is among the oldest colorant known to man.[11, 31] The shrub is approximately 2 to 5 meters tall and is native to the American tropics. Many tropical countries such as Bolivia, Brazil, Ceylon, Dominican Republic, Ecuador, Guyana, India, Jamaica, Mexico, Peru and Surinam now grow Bixa orellana. The fruit is found in a burr-like pod containing 10 to 50 seeds about the size of grape seeds. The seeds are covered by a thin layer of soft, sticky bright red pulp. The pulp contains the annatto pigment.

Annatto extracts may be water or oil soluble or suspensions of the pigment in oil. Details of the extractions and chemistry of annatto are provided by Preston and Reith[31,32]. In general, mechanical friction in conjunction with solvents such as vegetable oil, fats, alkali and alcoholic solutions are used to leach the color from the pulp.[15] The extract then is further refined depending on the final application. Precipitation with acids, recrystallization and spray drying in either oil or water soluble forms are possible processes.

The major pigments comprising annatto are the C_{25} diapocarotenoids bixin and norbixin in the cis and trans forms (Figure 2).[11, 15, 37] The major pigment (+80%) of the seed coat is bixin ($C_{25}H_{30}O_4$), the mono-methyl ester of the dicarboxylic acid carotenoid.[25] Stable forms of bixin were first isolated in 1913.[11] The two forms of bixin originally were referred to as labile/stable or α/β. Later research found the labile/stable or α/β forms were actually cis/trans isomers of bixin.

Bixin is soluble in fats and oils at < 0.1% by weight while the potassium or sodium salt of norbixin is soluble in water.[25] The color of bixin is dependent on pH ranging from yellow-orange to pink at lower pHs although pH does not affect the stability of the color. Bixin is stable at temperatures less than 100^{0}C (212^{0}F) but relatively unstable at temperatures greater than 125^{0}C (257^{0}F). The pigment also is unstable to light. *Cis*-bixin is orange in color and insoluble in vegetable oil. Heating converts *cis*-bixin into the isomer *trans*-bixin which is red and soluble in oil.

Norbixin is formed when the methyl group of bixin is saponified under alkaline conditions. The use of excess alkali causes the pigment to form an alkali metal salt that is soluble in water. When the saponified form is placed in an acidic environment the molecule can convert to the insoluble dicarboxylic form and precipitate. Emulsifiers may be used to prevent precipitation. Calcium in hard water and cheese also can be exchanged for the potassium associated with the saponified form leading to precipitation of the pigment. Norbixin also will react with protein to form a peach-red color.[25]

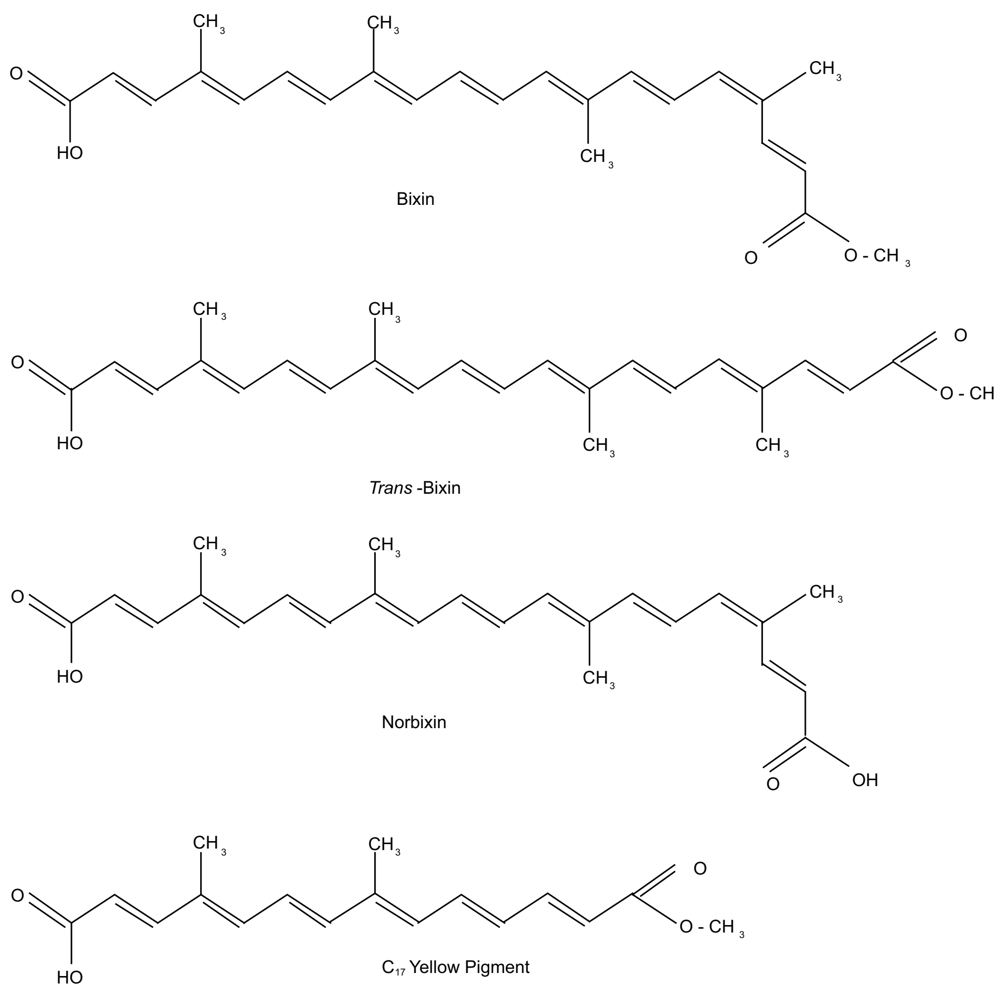


Figure 2. Structure of bixin, norbixin and C_{17} pigment.

Bixin and norbixin occur naturally in the cis form. Light and heat are the two main ways of changing unstable *cis*-bixin to the more stable *trans*-bixin form. A yellow pigment referred to as C_{17} ($C_{17}H_{20}O_4$) (Figure 2) results when bixin is heated. The cis forms of either pigment are more red than the trans forms. The carboxylic acid portion of the molecule contributes to water solubility while the ester form gives solubility in oil. Small amounts of other carotenoids and bixin degradation products are also present in annatto.

Commercially available annatto is considered to be a generally microbiologically clean product and resistant to microbial growth.[25] Annatto is purchased based on the percent bixin (*cis*) content or "points". Color will vary depending on variety of plant, growing area, climate/weather conditions, manufacturing methods and storage conditions of the seeds. High performance liquid chromatography can be used to determine the composition of annatto coloring agents.[35,37]

Oxidation of annatto is very important to the whey industry. Oxidation leads to loss of color by annatto. Oxygen is required and light acts as a catalyst for the reaction. Higher temperatures, presence of metal ions, greater intensity of light and greater availability of oxygen increase oxidation of annatto and loss of color. Some researchers have concluded that light was the most effective agent for causing loss of annatto color followed by benzoyl peroxide.[29] Contact with air is not considered very effective for oxidizing annatto. The presence of an antioxidant such as ascorbyl palmitate protects annatto from loss of color in the presence of light.

Carotenoids are known to combine with proteins to stabilize the carotenoid molecule.[15] In addition, annatto will react with carboxyl groups.[11] Because norbixin contains a carboxyl group the molecule can complex with divalent metal ions. Norbixin then is able to bind with the carboxyl group of another molecule thereby forming a stable complex. Such a complex can protect norbixin from oxidation and help retain the original color. While such a reaction may be desired for some products it may be a problem when color removal by bleaching is desired.

Annatto can produce a pink color in cheese. Some researchers have concluded that the pink color is the result of hydrogen sulfide involvement in the precipitation rather than oxidation of norbixin.[16] Other researchers have attributed the pink or peach-red color to a reaction of norbixin with protein.[25] The pink color may be protected from further color change by phospholipids and β-casein.[16] The pink color is stable to oxygen, light and pH changes.

Annatto also can cause a pink color in products containing bleached whey powder. Whey that previously has been bleached white and dried can become pink when incorporated into other products such as ice cream. The precise cause of the pink color is not known but factors such as pH, heat treatments to the whey and brand of annatto used are believed to be important in determining whether the defect occurs.

Although annatto in cheese largely is combined with casein, a portion of the annatto will be present in the resulting whey.[3, 7, 11] Early research with the water soluble form of annatto indicated approximately 10% of the added annatto went into the whey rather than into the cheese. Later work indicated 12 – 22% of the annatto partitioned into the whey.[4] More recent research found 18 to 26% of the bixin added to cheese milk is present in the resulting whey.[10] Lower levels of bixin addition (0.11 mg bixin/kg milk) had slightly higher percentages of bixin in the whey versus higher bixin addition levels (1.1 mg bixin/kg milk). The authors concluded that, in general, approximately 20% of the bixin added to cheese milk partitioned into the whey.

There is a lack of information on the status of norbixin in whey. Although norbixin will combine with casein it is not known whether norbixin has affinity for any specific whey components.

Some researchers have proposed that norbixin interacts with whey components other than proteins. Further research is needed in this area.

Annatto in whey powder is believed to be associated with the whey proteins.[2, 18] Researchers have dissociated the annatto from the whey proteins by using ammonia to make the rehydrated whey powder alkaline.[18]

Permitted Compounds

Hydrogen peroxide and benzoyl peroxide are the only compounds currently allowed in the United States for bleaching whey. Codex regulations recently have changed to permit the use of benzoyl peroxide for bleaching.

Hydrogen peroxide (H_2O_2) is a clear, colorless liquid having a slightly pungent odor and a molecular weight of 34.0. Food grade hydrogen peroxide typically has a concentration of 30 to 50%. Hydrogen peroxide is safe and stable under recommended storage and handling conditions, although hydrogen peroxide will decompose by exothermic reaction when exposed to soil and other foreign materials.

Benzoyl peroxide or dibenzoyl peroxide ($C_{14}H_{10}O_4$) is a colorless, crystalline solid with a molecular weight of 242.2. The structure of benzoyl peroxide is given in Figure 3. Benzoyl peroxide has a faint odor of benzaldehyde, is insoluble in water and melts with decomposition at temperatures of 103 to 106^{0}C (217 to 223^{0}F). The dry, concentrated form of benzoyl peroxide is a highly reactive, dangerous oxidizing material that may spontaneously explode. Commercial products may contain 15 to 35% benzoyl peroxide.

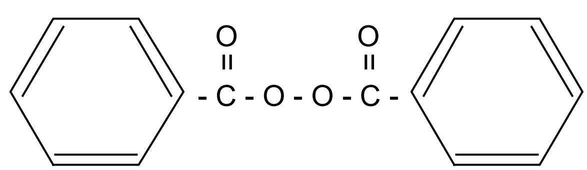


Figure 3. Structure of benzoyl peroxide.

Benzoyl peroxide for bleaching whey often is blended with carriers to facilitate use. The strength of the benzoyl peroxide and degree of water solubility will vary with the specific carrier used. Examples of carriers are: calcium sulfate, magnesium carbonate, starch, calcium phosphate and whey.

Conditions of Use

Although hydrogen and benzoyl peroxides may be used to bleach whey there are several restrictions to their use. The inherent advantages and disadvantages of each chemical may determine the suitability of either compound for a given application.

Hydrogen peroxide use as a bleaching agent is covered by 21CFR 184.1366. Hydrogen peroxide may be used at a rate of < 0.05% (< 500 ppm) and is considered effective at all temperatures and total solids levels. Residual hydrogen peroxide must be removed by appropriate physical and/or chemical means during processing. Addition of catalase to eliminate hydrogen peroxide is required according to FDA regulation 133.113. The amount of catalase added must be sufficient to eliminate any residual hydrogen peroxide and cannot exceed 20 ppm (0.002%) catalase based on the milk used. Hydrogen peroxide must meet the specifications of the Food Chemicals Codes. The only permitted use of hydrogen peroxide as a preservative is during electrodialysis where a maximum of 0.04% (400 ppm) hydrogen peroxide may be used. Advantages of hydrogen peroxide for bleaching include effectiveness over a wide range of temperatures and total solids levels. The corrosive nature of hydrogen peroxide, need to be inactivated with catalase, long hold times to remove color, and potential to cause oxidized flavors are disadvantages to its use.

Benzoyl peroxide use is permitted under 21CFR 184.1157 for removing color in whey both from naturally occurring colored compounds and annatto addition except in whey products for infant formula. Benzoyl peroxide for bleaching has no use rate limitation other than that dictated by good manufacturing practices. A typical use rate for benzoyl peroxide is < 0.002% (< 20 ppm). Most effective use conditions are 60^{0}C (140^{0}F) for 15 minutes at pH 6 to 7. Longer holding times are required if lower temperatures are used. Benzoyl peroxide is effective at lower usage levels, does not require catalase addition and does not pit stainless steel. There are several disadvantages to its use. Oxidized flavors can be produced by benzoyl peroxide treatments. Commercial benzoyl peroxide products use a carrier and the carrier may be considered an allergen. Use of benzoyl peroxide in products for export can be a concern. Although Codex regulations recently have changed to permit benzoyl peroxide as a bleaching agent some countries such as Japan still may be concerned about the use of benzoyl peroxide in imported products. Benzoyl peroxide will not reduce microbial populations or control acid production in whey.

Reactions and Conditions During Bleaching

Hydrogen and benzoyl peroxides have been used extensively for bleaching annatto. Many aspects of their general use have been determined, however, very limited supporting research is available to support some conclusions. A colorimetric method for measuring the amount of annatto and other related colors in dry whey has been developed to monitor the bleaching process and its effectiveness.[18]

Hydrogen peroxide decomposes to oxygen and water during bleaching (Figure 4). Excess hydrogen peroxide can be removed from the whey by addition of catalase. Heat also will eliminate residual hydrogen peroxide.

$$\underset{\text{Hydrogen peroxide}}{2H_2O_2} \longrightarrow \underset{\text{Oxygen}}{O_2} + \underset{\text{Water}}{2H_2O}$$

Figure 4. Breakdown reaction of hydrogen peroxide.

The ability of hydrogen peroxide to bleach annatto in Cheddar cheese whey is affected by temperature and presence of curd particles.[27] Reaction temperatures >74^{0}C (165^{0}F) do not increase either the rate or extent of color removal and instead cause protein denaturation. Oxidized flavors, apparent immediately after treatment, disappear following evaporation and drying.

Benzoyl peroxide reacts with oxidizable compounds and is converted into water soluble benzoic acid (Figure 5). The whey should not contain any benzoyl peroxide following bleaching as all of the benzoyl peroxide is converted to benzoic acid. Benzoic acid has been extensively studied as a food additive and may be used unconditionally at up to 5 mg/kg body weight. Benzoic acid has GRAS status for several food products.

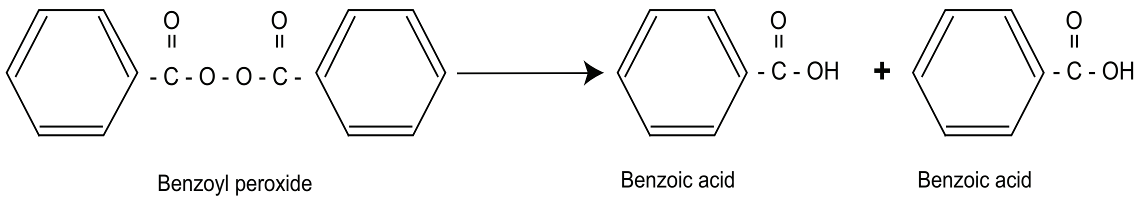


Figure 5. Breakdown reaction of benzoyl peroxide.

Benzoyl peroxide will bleach carotenoids present in cream.[23] Carotenoids were effectively destroyed by 0.0009% (9 ppm) benzoyl peroxide in cream at 125 to 145^{0}F (52 to 63^{0}C) for 1 to 2 hours. Vitamin A content of the cream was not significantly affected. Temperature affected the rate of color loss but not the final color rather concentration of benzoyl peroxide used determined amount of color loss. Temperatures of 165 to 185^{0}F (74 to 85^{0}C) resulted in cooked and scorched flavor while 0.0018% (18 ppm) benzoyl peroxide caused oxidized and tallowy flavors.

The effectiveness of benzoyl peroxide for removing color in whey depends on the amount of benzoyl peroxide used, how it is applied, whey components present, exposure time and temperature. A study comparing hydrogen and benzoyl peroxides found both were effective, however, benzoyl peroxide was more effective at all temperatures evaluated.[27] Oxidized flavors initially present dissipated following evaporation and drying.

Benzoyl peroxide readily removes annatto in solution.[2] When milk proteins are present annatto is more resistant to bleaching and additional benzoyl peroxide and/or time is required. Whey with higher total solids, such as condensed whey, also needs greater amounts of benzoyl peroxide to remove color. It should be noted that once whey has been dried, the annatto color becomes highly resistant to bleaching. The annatto is thought to be bound to proteins in the whey and once dried the reaction cannot be reversed to free the annatto for bleaching.

Color removal by benzoyl peroxide is very temperature dependent.[2] Recommended bleaching temperatures are 55 to 65^{0}C (131 to 149^{0}F). If temperatures are too low, residual annatto color may remain regardless of reaction time. For example, whey at 30^{0}C (86^{0}F) may never have the annatto color completely bleached.

Benzoyl peroxide reacts quickly to remove color.[2] Generally 30 minutes is sufficient to react all of the benzoyl peroxide with color compounds. Additional time will not increase color removal.

Optimum pH for bleaching is approximately 6 to 7.[2] Use of pHs below 5.5 are said to result in a more pink color. It may be that using benzoyl peroxide to bleach whey with a pH < 5.5 is a factor in the production of off-color whey.

Effectiveness Against Microorganisms

Research has focused primarily on the affects of hydrogen peroxide on microorganisms. Relatively little information is available on the interactions of benzoyl peroxide with microorganisms associated with milk and whey.

Hydrogen peroxide effectiveness in inactivating microorganisms depends on hydrogen peroxide concentration, microbial population, temperature, pH, inorganic ions, treatment time and exposure of microorganisms to additional treatments (chemicals, heat treatments, etc.).[5, 34] Oxidation and changes in the DNA and enzyme systems of the microorganisms are believed responsible for inactivation of the microorganisms although details are not known. The hydroxyl radical formed during the breakdown of hydrogen peroxide is thought to be involved in the killing of bacteria.[6] The hydroxyl radical may be the strongest oxidant known. Hydrogen peroxide is more effective in inactivating spores than bacterial cells.

Hydrogen peroxide is most effective against anaerobic sporeformers and coliforms.[6, 24, 34] Anaerobes do not produce catalase to break down hydrogen peroxide. Lactic acid starter bacteria used in cheese manufacture can be inhibited by hydrogen peroxide concentrations as low as 5 ppm (0.0005%) although they may be considered intermediate in their susceptibility to inactivation by hydrogen peroxide. Aerobic sporeformers are most resistant to hydrogen peroxide. Gram-negative bacteria are more sensitive to hydrogen peroxide as compared to gram-positive bacteria. Lipases and proteases in milk are not considered susceptible to inactivation by hydrogen peroxide.

Effectiveness of hydrogen peroxide for inactivating spores depends on temperature, pH, hydrogen peroxide concentration and number of spores.[14] In general, time required for inactivation of spores increases with increasing concentration of spores, decreasing concentration of hydrogen peroxide, increasing pH and decreasing temperature.

Studies determining the effectiveness of hydrogen peroxide for inhibiting microorganisms have generally used milk rather than whey as the substrate. Because milk is a more complex media than whey, it should be possible to extrapolate from studies using milk to studies using whey.

Lactic acid bacteria used for the production of cheese vary in their sensitivity to hydrogen peroxide.[38] One study found *Streptococcus lactis* and *Streptococcus diacetylactis* were able to produce acid in the presence of 80 µg H_2O_2/ml milk (80 ppm or 0.008%) after 6.0 hours of incubation in milk at levels similar to the same bacteria in milk without hydrogen peroxide. *Streptococcus cremoris* by comparison, was more sensitive to the presence of hydrogen peroxide. The affect of increasing hydrogen peroxide concentrations on inhibiting acid production was not linear and was affected by exposure time and the specific lactic acid bacteria present. In all cases, acid production during exposure to a given concentration of hydrogen peroxide was greater after 6 hours of incubation as compared to 3 hours regardless of the specific lactic acid bacteria present. Above a certain concentration of hydrogen peroxide no further reduction in acid production occurred. The hydrogen peroxide concentration where further retarding of acid production occurred depended on the specific lactic acid bacteria used. In general, hydrogen peroxide levels greater than 120 µg/ml (120 ppm or 0.012%) had little additional affect on reducing acid production.

Another study found similar results.[34] *Streptococcus lactis, Propionibacterium shermanii* and *Lactobacillus bulgaricus* were more sensitive to the presence of hydrogen peroxide (130^{0}F or

54^{0}C for 1 minute at 0.06% or 600 ppm hydrogen peroxide) than *Streptococcus thermophilus* and *Streptococcus diacetilactis*. Different strains of S. *diacetilactis* were more or less sensitive to inactivation by hydrogen peroxide.

Some microorganisms, including some starter bacteria, produce hydrogen peroxide that inhibits other microorganisms. *Streptococcus mitis* and *Lactobacillus acidophilus* are examples of such types of bacteria.

Some microorganisms produce catalase which can destroy hydrogen peroxide.[36] Bacteria that can produce catalase when grown in milk include: *Micrococcus spp., Staphylococcus spp., Bacillus spp., Corynebacterium spp.* and *Propionibacterium spp.. Lactobacillus and Streptococcus spp.* do not commonly produce catalase.

Bacteriophages of *Streptococcus cremoris* inoculated into milk were not inactivated by exposure to 10,000 ppm (1%) hydrogen peroxide at 54^{0}C (129^{0}F) for 1 hour. *S. cremoris* cells, however, were destroyed after 5 minutes exposure to 1,000 ppm (0.1%) hydrogen peroxide.[21] Milk apparently provided a protective affect since use of phosphate buffer in place of milk as a substrate led to inactivation of the bacteriophage.

Benzoyl peroxide decomposition is not significantly affected by the pH of whey.[9] Benzoyl peroxide also is considered stable to heat treatments.

Studies have found benzoyl peroxide concentrations of up to 50,000 ppm (5%) are not effective in significantly reducing the acid producing activity of some lactic cultures.[40] Benzoyl peroxide, however, is often used in other products to limit microbial growth. A possible explanation for the lack of antimicrobial activity is the typical pH of whey. Benzoyl peroxide breaks down to benzoic acid. Benzoic acid in both the dissociated and undissociated forms inhibits microorganisms, however, optimum antimicrobial activity for benzoic acid is between pH 2.5 to 4.0. The pH of whey almost always is above pH 4.0 therefore benzoyl peroxide/benzoic acid is ineffective at controlling growth of bacteria.

Affects on Whey Functionality/Flavor

The color of the whey is an important product characteristic, however, functional properties and flavor may be of even greater importance to the end user. The use of peroxides can alter these properties.

Hydrogen peroxide can alter the functionality of whey proteins.[13, 28] The susceptibility of whey proteins to hydrogen peroxide depends on the specific protein, concentration of hydrogen peroxide, temperature, time and pH.

Hydrogen peroxide is known to inhibit browning in milk systems.[30] Additional information on the mechanism of browning inhibition is lacking, however, it is likely hydrogen peroxide alters the ability of reactive groups on the proteins to interact with sugars thereby limiting the Maillard reaction.

When added to milk, hydrogen peroxide increased the amount of non protein nitrogen present while decreasing the concentration of whey proteins.[17] Increasing the concentration of hydrogen peroxide and contact time increased the affect. Proteose peptones were the most susceptible to denaturation by hydrogen peroxide. Immunoglobulins, bovine serum albumin and β-lactoglobulin were intermediate in susceptibility while α-lactalbumin was relatively unaffected by hydrogen peroxide treatments. Whey proteins were more susceptible to denaturation by hydrogen peroxide than casein. Hydrogen peroxide did not cause interactions between β-lactoglobulin and κ-casein.

Another study evaluated whey proteins after contact with various concentrations of hydrogen peroxide for 3 days at room temperature.[28] Hydrogen peroxide concentrations greater than 0.1% (1000 ppm) caused a 5 to 8% decrease in the non-polar amino acids. Amino acid residues affected included aspartic acid, threonine, glutamic acid, methionine, tyrosine, phenylalanine, histidine, lysine, tryptophan and arginine. Tryptophan with approximately 25% decrease in concentration, was most affected by exposure to hydrogen peroxide concentrations greater than 0.1% (1000 ppm).

The number of free sulphydryl groups increased with increasing hydrogen peroxide concentration. Increasing the storage time beyond 24 hours did not significantly increase the number of free sulphydryl groups. Lower concentrations of hydrogen peroxide did not cause significant oxidation of the sulphydryl groups; however, higher concentrations of hydrogen peroxide rapidly increased sulphydryl group oxidation. The researchers postulated that hydrogen peroxide reacts first with readily oxidized amino acids such as methionine. The structure of the amino acid is altered such that the sulfhydryl groups are exposed and then oxidized.

Hydrogen peroxide concentration, temperature and time were very important variables affecting whey protein denaturation. As expected, higher concentrations of hydrogen peroxide, increased temperatures and longer holding times all increased the amount of whey protein denaturation. Temperature had a very large affect on whey protein denaturation.

The pH resulting in the greatest amount of protein denaturation depended on the specific whey protein although the affect of pH was minor compared to the other variables. For example, immunoglobulins and bovine serum albumin were more readily denatured at lower pHs while β–lactoglobulin denaturation was enhanced at pHs closer to neutral. Alpha-lactalbumin was unaffected by comparison.

Researchers found whey at 25^{0}C (77^{0}F) with 0.5% (5000 ppm) hydrogen peroxide had minimal whey protein denaturation. Alternatively, approximately 1% (10,000 ppm) hydrogen peroxide at and 55^{0}C (131^{0}F) caused significant whey protein denaturation. Other researchers found hydrogen peroxide concentrations greater than 0.1% resulted in formation of methionine sulfone and cysteic acid that potentially could reduce the nutritional value of the whey proteins.

Benzoyl peroxide combined with heat also affects whey proteins.[40] Beta-lactoglobulin, α-lactalbumin, proteose peptone, serum albumin and immunoglobulins were affected with 1 and 5% (10,000 and 50,000 ppm) benzoyl peroxide addition yielding the most apparent changes. The affect of benzoyl peroxide on casein was less apparent.

One study evaluated the affects of bleaching with benzoyl peroxide on the foaming properties of whey protein isolate produced from sweet whey.[20] They concluded that foaming was not affected by either the annatto addition or bleaching by benzoyl peroxide although there were slight differences in non protein nitrogen, true protein and ash content.

Benzoyl peroxide can oxidize milkfat resulting in tallowy, oxidized flavors.[28, 40] Flavor problems are more apparent with increasing temperature, contact time and benzoyl peroxide concentration.

Additional Comments and Concerns on Peroxide Use

Several additional factors may need to be considered when deciding whether to use either hydrogen or benzoyl peroxides. Peroxide limitations, regulatory issues and international concerns may preclude the use of peroxides.

Peroxide limitations

Peroxide limitations affect the ability of either hydrogen or benzoyl peroxides to bleach whey. As with any food ingredient, only food grade hydrogen or benzoyl peroxides should be used. Because less concentrated hydrogen peroxide is prone to decomposition, concentrated hydrogen peroxide (> 35%) should be diluted to useable strength immediately before use. Contact with polyvalent metals such as copper and iron also will accelerate the decomposition of hydrogen peroxide.[12]

Naturally occurring enzymes present in milk and whey, such as catalase and peroxidase, can decompose the hydrogen peroxide added to remove color or control microbial growth.[1, 12] Increasing temperatures increases the efficiency of hydrogen peroxide for inactivating microorganisms; however, the rate of hydrogen peroxide decomposition also increases.

An example is a study where hydrogen peroxide was added to milk containing catalase and peroxidase. Storage at 135^0F (57^0C) resulted in relatively little hydrogen peroxide decomposition as compared to 100^0F (38^0C). Researchers believed enzymes in the milk decomposed the hydrogen peroxide when incubated at 100^0F (38^0C) while the enzymes were inactivated by storage at 135^0F (57^0C) resulting in stable hydrogen peroxide concentrations. Other researchers have noted that peroxide was more effective at temperatures above those typically used during starter culture growth in cheese manufacture.[34]

Although peroxide will lighten coloring compounds such as annatto added to milk, peroxide will not remove brown color compounds resulting from caramelization or Maillard reactions. Hydrogen peroxide can inhibit browning but it cannot alter brown color compounds after their formation.

Additional information is available from studies that have focused on hydrogen peroxide use in milk as an alternative preservation method. Hydrogen peroxide can oxidize proteins, aldehydes, ketones and vitamins A and C in milk.[36] In addition, hydrogen peroxide can split the $\alpha_{S1\text{-}5}$ fractions from casein micelles resulting in longer clotting and setting times and softer curd during cheese manufacture.

Regulatory concerns

Regulatory concerns focus on the use of either hydrogen or benzoyl peroxide for preservation of whey. As previously discussed, the use of peroxide for preservation of whey during any process other than electrodialysis is prohibited. Regulatory agencies typically use the point of peroxide addition in the process to determine if the purpose of peroxide is bleaching or preservation.[39]

The bleaching of whey generally is done at one of two locations in the process. One location is when preheated whey is pumped into a storage tank and peroxide then added. The second location is addition of peroxide at the hot well of the evaporator. USDA cites two specific cases where use of peroxide would be assumed for preservation purposes.[39] The first case is addition of peroxide before the separator or any point in the process before preheating for the evaporator. The second location generating concern is addition of peroxide before holding the whey for more than two hours at temperatures between 45 and 145^0F (7 and 63^0C).

Catalase or hydrogen peroxide oxido-reductase is an enzyme used to remove hydrogen peroxide. Catalase converts hydrogen peroxide to oxygen and water (Figure 6). Crystalline catalase originally was isolated from beef liver in 1937 although catalase also is present in high concentrations in blood, liver, kidney and fatty tissues.[34] The enzyme has a molecular weight of 225,000 daltons and it is considered to be relatively stable and very active. One molecule of catalase can decompose 2,600,000 molecules of hydrogen peroxide at 0^0C (32^0F) in one minute.

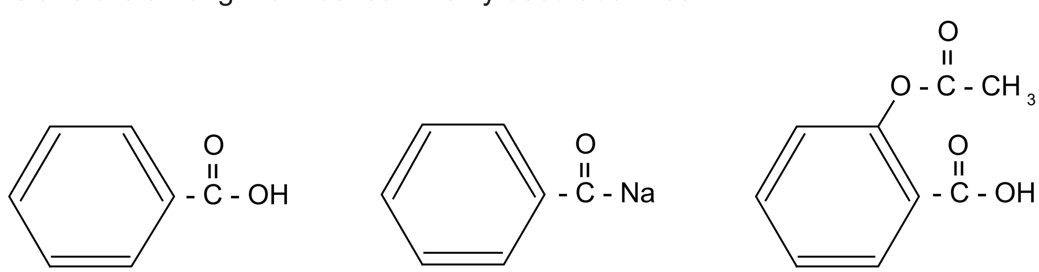
$$\underset{\text{Hydrogen peroxide}}{2H_2O_2} \longrightarrow \underset{\text{Oxygen}}{O_2} + \underset{\text{Water}}{2H_2O}$$

Figure 6. Reaction of catalase and hydrogen peroxide.

Commercial catalase is a fermentation product of *Aspergillus niger.* In liquid form, catalase is amber to dark brown in color with a fermentation odor. As a powder catalase has a tan color. Catalase is completely soluble in water. The enzyme typically is active from pH 2 to 7 and 0 to 65^0C (32 to 149^0F) and should be stored in a cool, dry location.

International concerns center on the use of benzoyl peroxide. Many Asian and European countries do not like the use of benzoyl peroxide although CODEX recently approved both hydrogen and benzoyl peroxide for use in bleaching whey.

The presence of benzoic acid is an issue for certain markets. The major decomposition product of benzoyl peroxide is benzoic acid.[9] The safety of benzoic acid and its derivative benzoates (Figure 7), has been widely studied.[26] Benzoates and the related salicylates are widely distributed in food plants and are present in prunes, tea, cloves, cinnamon and many berries. Benzoic acid and benzoates have been used as preservatives in food and beverages for approximately 100 years and are among the most commonly used additives.



Benzoic acid | Sodium benzoate | Aspirin

Figure 7. Structures of benzoic acid, sodium benzoate and aspirin.

Originally it was believed that benzoic acid related compounds did not cause adverse reactions when consumed. It is now apparent that a small percentage of the population is sensitive to such compounds.[26] People with adverse reactions to benzoic acid related compounds typically have underlying diseases such as asthma. Asthmatics often are intolerant to aspirin ($C_9H_8O_4$), also known as 2-acetoxybenzoic acid and O-acetylsalicylic acid, which is very similar in structure to benzoic acid. The mechanism of the intolerance does not appear to be an allergy type but rather a pseudo-allergic response that relies on enzymes rather than an immunological reaction.

Adverse reactions to benzoic acid related compounds appear to be relatively rare.[19] An exception would be asthmatics with aspirin intolerance. Reactions generally are mild with life-threatening reactions extremely rare.

Additional Sources of Benzoic Acid

Benzoic acid is produced by lactic starter cultures. Unfortunately the presence of benzoic acid due to microbial activity can cause concerns in some export markets where it may be incorrectly assumed that bleaching of the whey product has occurred despite assurances to the contrary by the producer.

Benzoic acid occurs naturally in low levels in milk.[8] Several strains of lactic acid bacteria are able to convert hippuric acid ($C_9H_9NO_3$), a natural constituent of fresh milk, to benzoic acid during fermentation of milk. The structure of hippuric acid, also known as N-benzoylglycine and benzoylaminoacetic acid, is given in Figure 8.

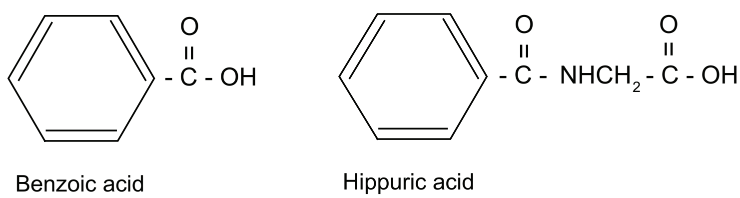


Figure 8. Structures of benzoic acid, hippuric acids.

A few studies have determined the levels of benzoic acid in dairy products.[8, 22, 33] A summary of the results is given in Table 1. Considerable variability in benzoic acid content was possible for a given product. One group attributed the variability in benzoic acid concentration to factors such as method of acidification (fermentation or direct acid addition), variations in the manufacturing procedures, natural variation in the hippuric acid content of milk and degree of hippuric acid hydrolysis.[33]

Product	Benzoic acid content (ppm)
Raw whole milk	Trace
Raw skim milk	Trace
Paseurized whole milk	4-6
Fermented skim milk	30-60
Sour cream	11-18
Buttermilk	11-20
Yogurt	16-56
Cottage cheese	9-18
Cheddar	17
Mozzarella	26
Variety of cheeses	Trace-35

Table 1. Benzoic acid content of dairy products [8, 22, 33]

The partitioning of benzoic acid during cheese, whey and whey protein concentrate production also has been studied.[3, 8] Figure 9 is an outline of the development and distribution of benzoic acid during production of cottage cheese cultured with lactic acid bacteria. Benzoic acid partitioned with the serum portion of the milk and was present in the whey when drained from the curd. During manufacture of lactic acid casein, a product produced using methods similar to those used for cottage cheese, washing the curd removed 90% of the benzoic acid present. Essentially 90% of the benzoic acid produced therefore was present in the acid whey. When the acid whey was processed into a whey protein concentrate, the researchers found 95% of the benzoic acid passed into the permeate.

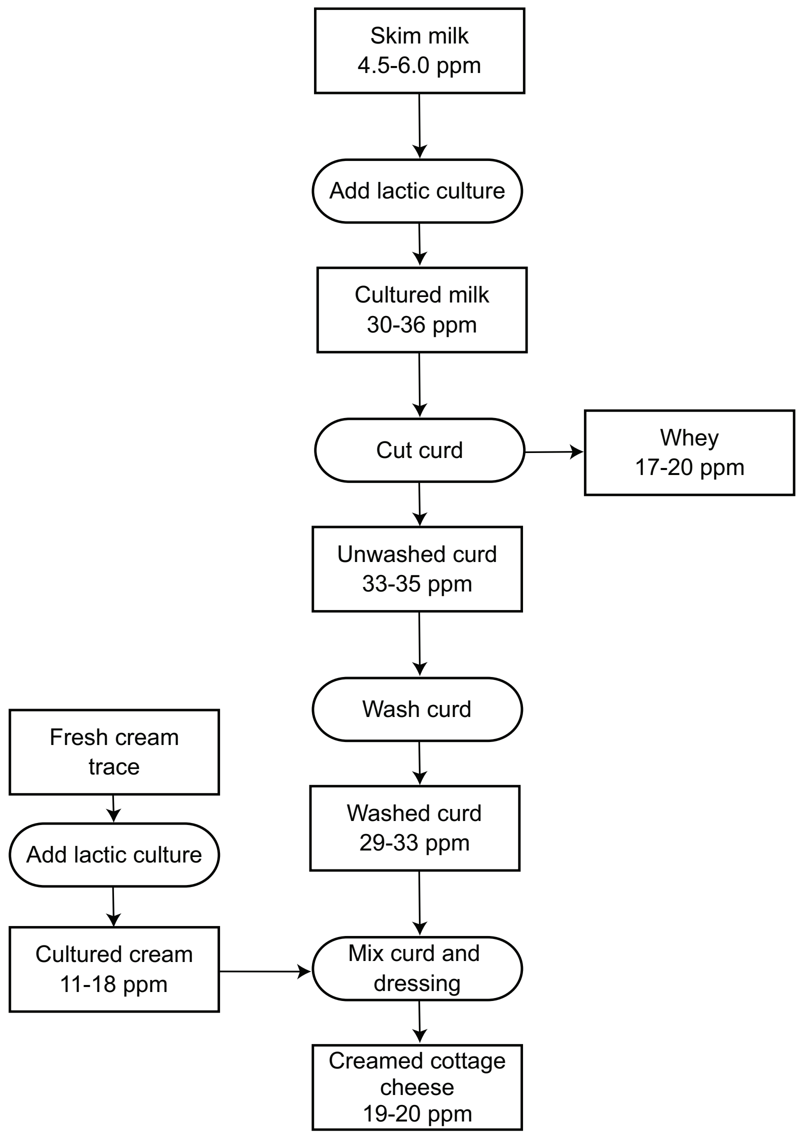


Figure 9. Outline of benzoic acid distribution in cottage cheese.[8] Benzoic acid concentration is given ppm.

It is very probable that the benzoic acid content of a whey product will depend on the amount ultrafiltration and diafiltration used in processing. Whole whey from a lactic acid bacteria cultured cheese should contain significantly more benzoic acid than a whey protein concentrate produced from the same whey. Also, a WPC80 should have less benzoic acid than a WPC34 from the same whey since the WPC80 will have had greater removal of permeate. Only trace amounts of benzoic acid should be present in whey products produced by direct acid addition of the milk.

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