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DEOXYGENATING METHOD AND PRODUCT

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This invention relates to a method of protecting materials from the deleterious effects of free oxygen. More particularly, it relates to stabilization of articles of manufacture by removing free oxygen from association with the articles in relatively gas tight receptacles. Still more particularly, it relates to a dry pulverulent material comprising a solid carrier or base, glucose and a nonviable enzyme system having glucose oxidase activity, which may be intermingled with the dry product to be protected or merely associated therewith in an enclosed space but separate from the product to be protected by a gas permeable barrier.

This application is a continuation-in-part of our application Serial No. 686,881, filed September 30, 1957, now U.S. Patent No. 3,016,336, entitled Deoxygenating Method and Product.

Numerous types of industrial products are adversely affected by free and uncombined oxygen. Oxygen reacts with these products with varying degrees of speed depending upon their chemical nature. The deleterious effects of reaction with oxygen generally show up more rapidly in foods, solid or liquid, in which the oxidative deterioration results in impairment of flavor, alteration of color, destruction of vitamin content and the like. These reactions, with some types of food, can be so rapid that they occur within ten minutes of exposure and before complete processing can be effected.

Slower but nevertheless destructive oxidative reactions occur in other fields, for example, the corrosion of metals. Materials of construction of the food containers themselves can be a problem. In the absence of suitable linings and coatings, the metal container may be attacked and become perforated. Other types of metallic objects, particularly those which are basically ferrous metal likewise require elaborate methods of protection during shipping and storage to prevent corrosion even though enclosed in plastic cocoons or other types of protective packaging.

Elimination of the oxygen from the receptacles or containers has been suggested for foods held in hermetically sealed cans. Oxygen elimination has been accomplished by enclosing in the container, but out of contact with the food, a moistureproof but oxygen permeable bag containing an aqueous dispersion of glucose and an enzyme system having glucose oxidase activity. Use of a moistureproof bag has been deemed necessary heretofore because an aqueous substrate is required for the enzymatic reaction to occur. The disadvantage of this separate bag has been that the films, which are sufficiently impermeable to moisture to hold the aqueous dispersion, also are very low in their gas or oxygen permeability and retard the oxygen reaction making oxygen removal a slow process.

When a material, normally subject to oxidative deterioration, can be isolated in a confined space or closed receptacle the amount of free oxygen present is limited to the amount absorbed in the material to be protected, the amount contained in the air about said material and that amount which may enter the confined space by permeation of the space walls or through leakage. In the normal course of industrial operation, the total amount of oxygen to be removed for any particular type of packaging arrangement is determinable.

The present invention contemplates the use of a solid deoxygenating body comprising a solid carrier or base, preferably of porous character, having deposited therein

and thereon a deoxygenating composition which is an enzyme system reactive with oxygen in the presence of substrate and containing water sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water. If the elimination of by-products such as hydrogen peroxide, formed during oxidation, is necessary for completeness of oxygen removal then additional enzymes may be introduced to convert the harmful by-product to a harmless one. For example, hydrogen peroxide may be eliminated by any one of a number of means, preferably by the use of catalase.

Solid material useful as a carrier or base may vary as to chemical character, i.e., be organic or inorganic chemical compositions; and may vary as to physical character, i.e., particle size and porosity. Depending upon the use, the base may vary from a food ingredient to blocks of gypsum or pottery shards. When the deoxygenation is for the protection of dry foods adapted to either human or animal consumption, the carrier may be the entire food compositions or a portion thereof of an ingredient of the food composition and be consumed therewith. In the case of animal foods, the carrier, preferably in a comminuted form, may be corn meal, corn cob, sugar cane bagasse, beet pulp residue, sawdust and similar materials to give but a few examples.

Under other circumstances, the carrier plus enzyme may be intermingled with the food product subject to removal before use, by suitable separation means such as screening. When the deoxygenating body is to be separated from materials with which it is intermingled, the choice of particle size of the particulate body will be determined by the particle size of the material with which it is intermingled. The body may vary from a size requiring accurate screening for separation to a size manually removable. For example, the material subject to oxidative deterioration is a powder smaller than 20 mesh standard screen size, the carrier may vary from larger than 10 mesh size to a single unit having many square inches of surface area.

If the deoxygenating body or oxygen scavenger is to be kept isolated at all times, the particle size of the carrier will at least in part be determined by the character of the restraining barrier. Under these circumstances, the barrier may be formed of suitable construction material, permeable to oxygen or nonpermeable material having perforations therein. Oxygen-permeable materials may be paper such as is used for enclosing tea, soluble coffee and the like or woven fabrics whose filament size and weave determine the interstitial voids. Barriers perforated to give oxygen access to the enzymatic composition usually are of a metallic or synthetic resin composition.

Film materials which are permeable to oxygen and slowly permeable to moisture may also be used, for example, polyethylene film of the order of ½ mil thickness and foamed resin sheets having continuous passageways formed therein.

Sizes of perforations usually are governed by particle size of the two materials being kept isolated. Carrier base particles utilized for formation of the deoxygenating bodies can always be chosen of a size to be retained by the barrier material. The barrier material therefore is chosen to be such as to restrain the smaller particle size material from passing into the space reserved for the larger particle size material. In general, the particle size of the carrier can vary from blocks having many square inches of surface to comminuted material of a particle size all of which will pass through a 100 or 200 mesh standard screen. The need for appreciable surface area for activity or reaction makes comminuted or pelletized

carriers having a particle size in the range of 30 to 80 mesh preferable for many industrial uses.

In carrying out this invention, in a preferred embodiment thereof, a deoxygenating body is prepared by impregnating or otherwise depositing on and in the carrier or base an enzyme system containing glucose oxidase with or without catalase, preferably with catalase, in a phosphate buffered aqueous solution containing glucose. In accordance with this invention, glucose oxidase and a glucose substrate have been referred to as being important ingredients of the deoxygenating body. However, other oxidases or dehydrogenases that are capable of catalyzing a reaction between molecular oxygen and a specific substrate for the particular oxidase or dehydrogenase in an aqueous medium may also be employed. Thus, molecular oxygen will combine with (1) phenols and catechols in the presence of tyrosinase; (2) aldehydes and purines in the presence of aldehyde-oxidase; (3) amino acids in the presence of amine acid oxidase; (4) uric acid in the presence of uricase; (5) mannose or galactose in the presence of mannose oxidase or galactose oxidase; (6) monoamines and diamines in the presence of amine oxidase and (7) unsaturated fatty acids in the presence of lipoxidase, and the like.

The enzyme preparation containing oxidase and catalase or other suitable deoxygenating materials may be prepared in accordance with known procedures. If a commercial type of glucose oxidase enzyme is used, it may be desirable to remove materials which inhibit the activity and/or stability of the deoxygenating body impregnated therewith. Treatment with ion exchange, adsorbent or absorbent material such as the dextran polymer known as "Sephadex" may be used to remove the inhibitory material.

Moisture content of the carrier or base will be determined, in general, by its physical character, processing treatment and the like. In use, it is desired that the deoxygenating body have sufficient moisture to support enzyme activity but remain superficially in a dry state, i.e., the surface be devoid of free and unbound water which will cause agglomeration, balling and the like. Many of the otherwise suitable carriers contain insufficient moisture. This deficiency may be corrected by direct addition of water or by adjustment of the water content of the oxidase dispersion which is deposited on the carrier. Clay materials such as fuller's earth will absorb their weight or more of water without becoming surface wetted to cause agglomeration. These clays therefore in powder or pelletized form provide an excellent ingredient for blending with other carriers to increase the moisture held by the surface dry carrier material. In general, it is preferred to maintain a moisture content for the deoxygenating body consisting of carrier and enzyme system of between about 7% and about 50% by weight. In general, with moisture contents within this range, the activity of the deoxygenating composition over a limited period of time will decrease as the moisture content is lowered. Subjecting the carrier, interpenetrated with aqueous dispersion of glucose-enzyme system, to vacuum treatment at temperatures lower than about 45° C. for periods of varying length provides means for control over the moisture content of the deoxygenating body. Moisture content within a closed receptacle must be kept sufficiently low not to permit diffusion of excessive amounts of water vapor to the dry product subject to oxidative deterioration. Where even the passage of small quantities of water vapor out of the enzyme product are undesirable a desiccant may be used in conjunction with said deoxygenating body.

It will be apparent that the size of the deoxygenating body and the concentration of the various ingredients therein may be varied widely and will be dependent in part upon the amount of oxygen that must be removed from the enclosed space. The rate at which the enzyme material herein discussed will take up oxygen is a function of temperature, concentration of enzyme and active surface area of the deoxygenating body exposed to the air. The amount of oxygen, that can be taken up in a given period of time, is limited by the amount of glucose to combine with the oxygen and the amount of buffer present to neutralize the acid formed.

The deoxygenating body in its preferred form, in addition to a buffer, may contain preservatives and thickeners. Thickeners generally are used in an amount only to increase the viscosity of the dispersion. The thickeners may comprise such substances as agar, gelatin, gums, carboxymethyl cellulose or inorganic material such as silica and the like. Suitable preservatives for the deoxygenating composition are sodium dehydroacetate, merthiolate and others which will stabilize against decomposition by microorganisms. Sufficient buffer, such as alkali metal salts of phosphate, acetate, gluconate and the like is usually added to prevent the pH of the deoxygenating body from falling below about 3 to 4 since the enzyme system may be adversely affected under such acid conditions. Calcium carbonate may be added to neutralize gluconic acid formed during the oxidation reaction.

The invention, in a preferred embodiment thereof, is carried out by preparing a phosphate buffered aqueous solution of glucose and sodium dehydroacetate. Enzyme system containing glucose oxidase and catalyase is dispersed in the glucose solution. The resulting dispersion may contain 5 to 45% glucose, up to 0.6% sodium dehydroacetate and 0.1 to 1000 units per milliliter of glucose oxidase. In the absence of catalase, oxygen removal is faster but is not as complete as has been hereinbefore described. The hydrogen peroxide-catalase reaction produces 1 mole of oxygen for each 2 moles of H_2O_2 decomposed. When catalase is present, the reaction formed oxygen must be removed by the glucose oxidase reaction as well as atmospheric oxygen. The dispersion is 0.05 to 1.0 molar with respect to sodium acid phosphate and the pH is adjusted to between about 5.5 and 7.5. If 20 cc. of this type dispersion containing 500 glucose oxidase units per cc. is atomized onto 20 grams of carrier base having a particle size of +10 −20 mesh, this deoxygenating body, when introduced into a closed container of approximately 250 cc. gas capacity connected by suitable tubing to a mercury manometer, will produce in approximately 15 minutes vacuum of about 2½ inches, i.e., removal of about 40% of the oxygen in the enclosed space. In applications where comparatively large volumes of oxygen are to be removed the enzyme body should be heavily buffered to prevent pH drop. About ½ gram of gluconic acid is formed in removing 50 ml. of oxygen from the gas and use of 1 gram of calcium carbonate would be sufficient to effectively neutralize the gluconic acid formed. The total amount of glucose present must be at least equal to the stoichiometric equivalent of the amount of oxygen to be removed.

In forming a deoxygenating body the carrier base may be interpenetrated or impregnated with enzyme composition by any suitable means. Atomizing accomplishes a wide distribution of an aqueous dispersion over a large surface area. Where particle size is large and where capillary action is insufficient to fully impregnate the carrier, the carrier may be impregnated by mixing with the aqueous system or submerging it in the aqueous system, removing the wetted solids from the aqueous system and subjecting it to vacuum and then releasing the vacuum. By this means the pores and capillaries may be filled with the enzyme system and the surface of the carrier develops an outwardly dry appearance.

Among the carriers that may be used are vegetable fibers and residues, such as sawdust, ground corn cobs, woody ring of corn cobs, bark, bagasse and the like; glasses and plastics, such as striated Foamglass, polystyrene, polyurethane compositions, and the like; powdered and molded clay products, such as bentonite, fire clay,

pottery shards and the like; natural and synthetic minerals, such as fuller's earth, asbestos, dolomite, calcium silicate and the like; inorganic salts, such as calcium carbonate, alumina, titanium oxide and the like; porous abrasives, such as pumice, talc, vermiculite and the like; animal by-products, such as leather flour, bone meal and the like; cereal grains in whole or comminuted form, such as wheat, barley, rice, corn and the like.

The invention will be more fully understood from the following examples:

Example 1

300 grams of glucose hydrate, 8 grams of sodium dehydroacetate and 178 grams of disodium phosphate duohydrate were dissolved in 1 liter of water to form a buffer solution. 150 ml. of this buffer solution were mixed with 150 ml. of dilute glucose oxidase solution containing approximately 60 units per ml. of glucose oxidase and approximately 20 units per ml. of catalase. This mixture was then mixed with 400 grams of 40/60 mesh ground woody ring of corn cobs. 22½ grams of the above carrier and enzyme were introduced into No. 2 size cans and the cans hermetically sealed. After 2 hours, puncturing of the cans showed development of a vacuum therein.

Example 2

A 150 ml. portion of the buffer solution prepared in Example 1 was mixed with 500 grams of a composition consisting of 400 grams of 40/60 mesh ground woody ring of corn cobs and 100 grams of calcium carbonate. 40 grams of the deoxygenating body were sealed in each of a number of No. 2 size cans. Into half of the cans, prior to sealing, were placed beakers containing a CO_2 absorber. The cans were hermetically sealed. The following day the cans were punctured. Cans which did not contain a CO_2 absorbent showed no vacuum since the evolution of CO_2 from the carbonate produced a gaseous replacement volumewise for the oxygen taken up in the glucose-enzyme reaction. Cans containing a CO_2 absorbent showed a vacuum of 5.5 inches of mercury measured by a manometer.

Example 3

20 ml. portion of the buffer solution prepared in Example 1 was mixed with 0.2 ml. of dilute glucose oxidase solution containing approximately 60 units per ml. of glucose oxidase and approximately 20 units per ml. of catalase. This aqueous dispersion of enzyme was mixed with 29 grams of comminuted woody ring of corn cobs. 16 grams of the deoxygenating composition consisting of base plus dispersion of enzyme contained only about 2.5 units of glucose oxidase and about 1 unit of catalase. 2, 8 and 16 gram portions of the deoxygenating body were sealed in 500 ml. Erlenmeyer flasks. After 16 hours the readings on mercury vacuum manometers were 0.2, .9 and 1.8 cm., respectively. After 36 hours the vacuum readings were 0.4, 1.3 and 3.2, respectively.

Example 4

A glucose buffer solution was prepared in the manner described in Example 1 having a composition consisting of 7.5% glucose, 0.4% sodium dehydroacetate. The glucose solution was adjusted to 0.25 molar phosphate concentration with disodium phosphate and phosphoric acid giving the solution a pH of approximately 6.5. 80 mls. of the buffer solution were mixed with 1.8 mls. of enzyme solution containing approximately 750 units per ml. of glucose oxidase and approximately 400 units of ml. of catalase. This dispersion was mixed with 40 grams of expanded vermiculite. The deoxygenating composition was placed in bags made up of heat-sealable paper. These bags were then placed in glass flasks which were sealed and secured to vacuum gauges. At the end of 24 hours the gauge showed 2 inch vacuum. At the end of 48 hours the gauge showed 4.3 inches of vacuum indicating removal of a substantial portion of the oxygen.

Example 5

A 20 ml. portion of the buffer solution prepared in Example 1 was mixed with 2.0 ml. of dilute glucose oxidase solution containing 60 units per ml. of glucose oxidase and approximately 20 units per ml. of catalase. This aqueous dispersion of enzyme was mixed with 29 grams of comminuted woody ring of corn cobs of 50/80 mesh particle size.

1 part by weight of this oxygen scavenger is mixed with 250 parts by weight of prepared guinea pig feed and the intermingled material pelletized by compression.

Pellets of feed were deposited in multi-wall bags and after storage, analysis for ascorbic acid showed excellent retention of ascorbic acid in an unoxidized state.

Example 6

A 20 ml. portion of the buffer solution prepared in Example 1 was mixed with 2.0 ml. of dilute glucose oxidase solution containing 60 units of glucose oxidase per ml. This aqueous dispersion of enzyme was mixed with 29 grams of fuller's earth. The resultant mixture was packaged in heat-sealing tea bag stock. This fuller's earth base scavenger was placed in 500 ml. Erlenmeyer flask attached to mercury manometer. After 24 hours the manometer showed four inchs of vacuum.

Example 7

A 6.3 ml. portion of the buffer solution prepared in Example 1 was mixed with 0.7 ml. dilute enzyme solution containing 60 units per ml. of glucose oxidase. This was then injected into a heat-sealed tea bag containing nine grams of whitepine sawdust of 60/80 mesh particle size. Upon manipulation of bag and contents, the free liquid was taken up by the carrier. One tea bag, thus produced, was enclosed in a 500 cc. container. After 24 hours, the container showed approximately 4 inches vacuum as measured by a mercury manometer.

It will be seen that means have been provided for removing uncombined oxygen from the interior of closed containers by utilizing a deoxygenating body with the packaged product. The term "packaged product" used herein is not intended to be restricted to anhydrous products but is intended to mean commercially dry or dehydrated products which may be used to designate powdered, granulated, granular or concentrated materials or non-aqueous materials or water-containing materials to which it is undesirable to add enzyme directly.

By the use of this invention no special means are required to evacuate or flush air from the package prior to closure. If the container is sealed with enclosed or entrapped air, the oxygen is gradually removed from such air by the reaction converting glucose to gluconic acid. Care should be exercised not to expose the deoxygenating body to atmospheric oxygen for extended periods prior to sealing the container because in the presence of sufficient moisture the body may be rendered ineffective for the intended purposes.

Although the invention has been described in connection with specific embodiments thereof, it will be understood that these are not to be regarded as limitations upon the scope of the invention except insofar as included in the accompanying claims.

We claim:

1. An article of manufacture which comprises a closed receptacle containing a product normally subject to oxidative deterioration, a solid carrier for enzyme, a deoxygenating composition interpenetrated into said solid carrier to form a deoxygenating body, said deoxygenating composition comprising substrate, an enzyme system reactive with oxygen in the presence of said substrate and water sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water.

2. An article of manufacture which comprises a closed receptacle containing a product normally subject to oxida-

tive deterioration, a solid carrier for enzyme, a deoxygenating composition interpenetrated into said solid carrier to form a deoxygenating body, said deoxygenating composition comprising glucose, a nonviable enzyme system having glucose oxidase activity and water sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water.

3. An article of manufacture which comprises a closed receptacle containing a product normally subject to oxidative deterioration, a solid carrier for enzyme, a deoxygenating composition interpenetrated into said solid carrier to form a deoxygenating body, said deoxygenating composition comprising between about 5% and about 45% by weight of glucose, a nonviable enzyme system having glucose oxidase and catalase activity and between about 7% and about 50% by weight of water, said amount of water being sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water.

4. An article of manufacture which comprises a closed receptacle containing a product normally subject to oxidative deterioration, a granular solid carrier for enzyme, a deoxygenating composition interpenetrated into said granular solid carrier to form a deoxygenating body, said deoxygenating composition comprising substrate, an enzyme system reactive with oxygen in the presence of said substrate and water sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water.

5. An article of manufacture which comprises a closed receptacle containing a product normally subject to oxidative deterioration, a pulverulent carrier for enzyme, a deoxygenating composition interpenetrated into said pulverulent carrier to form a deoxygenating body, said deoxygenating composition comprising substrate, an enzyme system reactive with oxygen in the presence of said substrate and water sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water.

6. The article recited in claim 1 wherein the deoxygenating composition includes a buffer to adjust the pH of the composition to between about 3.0 and about 7.5.

7. The method of removing free oxygen from contact with products normally susceptible to oxidative deterioration which comprises enclosing said product in a receptacle closed to prevent free ingress and egress of gaseous mediums containing oxygen, introducing into said receptacle a deoxygenating body comprising a solid carrier for enzyme having a deoxygenating composition interpenetrated into said solid carrier, said deoxygenating composition comprising substrate, an enzyme system reactive with oxygen in the presence of said substrate and water sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water.

8. The method of removing free oxygen from contact with dry particulate food products normally susceptible to oxidative deterioration which comprises intermingling said food product with a particulate deoxygenating body comprising a solid carrier for enzyme having a deoxygenating composition interpenetrated into said solid carrier, said deoxygenating composition comprising substrate, an enzyme system reactive with oxygen in the presence of said substrate and water sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water and packaging the intermingled particles in a closed receptacle.

9. The method of removing free oxygen from contact with products normally susceptible to oxidative deterioration which comprises enclosing said product in a receptacle closed to prevent free ingress and egress of gaseous mediums containing oxygen, positioning within said receptacle, a deoxygenating body separated from said product by a water and gas permeable barrier, said deoxygenating body comprising a solid carrier interpenetrated with substrate, an enzyme system reactive with oxygen in the presence of said substrate and water sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water.

10. The method of removing free oxygen from contact with products normally susceptible to oxidative deterioration which comprises enclosing said product in a receptacle closed to prevent free ingress and egress of gaseous mediums containing oxygen, positioning within said receptacle, a deoxygenating body separated from said product by a water and gas permeable barrier, said deoxygenating body comprising a solid carrier interpenetrated with glucose, a nonviable enzyme system having glucose oxidase activity and water sufficient to support oxidase activity while the outer surface of said carrier remains substantially free of unbound water.

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