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OXIDATION PROCESS

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This invention relates to improvements in oxidation products of organic compounds and in processes of preparing the same.

The present invention relates more particularly to improvements in the processes and products described and claimed in Patent No. 1,994,992, dated March 19, 1935. In that patent, processes are disclosed in which organic materials such as animal or vegetable fatty materials are treated with oxygen or gas containing free oxygen in the presence of enzymic material containing enzymes to effect the catalytic oxidation of the organic materials. Various fatty materials are mentioned, by way of example, as being readily oxidizable by this process such as for instance, coconut oil, cottonseed oil, corn oil, poppyseed oil, butter, lard, hydrogenated fats or oils, and fatty acids such as oleic acid, or esters derived from oils and fats and mixtures of the same. The present invention, however, provides for the use of certain steps described below, whereby fatty material is more readily oxidized and with greater efficiency and economy. It is noted that certain fatty materials, such as cottonseed oil, for instance, are more susceptible to catalytic oxidation in the presence of enzymes than other fatty materials, and that the more resistant oils are more readily oxidized by the process to be described. Peanut oil, which, so far as is known, has not heretofore been oxidized by others catalytically in the presence of enzymes, though highly resistant to oxidation under ordinary conditions, is readily oxidized by the present process with highly favorable results.

Certain factors which are found to be of importance in the present invention, are the manner of agitation of the enzyme material and fatty material in presence of the oxidizing medium; the condition of the fatty material; the condition and concentration of the enzyme material; the manner of bringing together the fatty material and enzyme material; and when water is present, the relative proportion of fatty material and the aqueous phase. These factors are discussed separately below.

Intimate contact of enzyme, fatty matter and oxygen (air) is essential to the success attained in the present invention. To bring about this intimate contact an agitator of the type in which air is taken into the body of the reacting mass by the mixing means may be employed. It is also possible to bring about mixing of the reaction mass by bubbling gas containing free oxygen therethrough.

The above type of apparatus may be provided with a tight-fitting cover. The free space above the liquid to be agitated may be filled with pure oxygen, or inert gases (such as nitrogen, etc.) more or less enriched with oxygen. During the reaction, the reaction chamber may, if desired, be kept under superatmospheric pressures. Pressures up to 100 pounds, for instance, have proved highly successful.

It is most convenient to use liquid fats for treatment. Solid fats should be first melted. It is preferred in the treatment of melted fats which are solid at ordinary temperatures, that the temperature of the reaction mass be kept a few degrees above the melting point of the fat treated.

The enzyme material is employed in the form of a dispersion preferably in aqueous media, or in media harmless to the enzymes or in other words, media that do not impair the enzymic activity required in the reaction. The dispersion is preferably a substantially clear solution or a clarified extract. In most cases it is a more or less turbid solution substantially free from visible particles. An extract is best obtained by treating flour or meal of soya beans or other leguminous substances including navy beans, green or yellow peas, and lentils, with a dilute solution of a calcium or magnesium salt. Solutions of calcium chloride of 0.1% to 0.2% strength are preferred, particularly for extracting enzymes from soya beans. Such a solution extracts the active enzymes rapidly and prevents certain troublesome proteins and gum-like substances from passing into solution. The latter substances are undesirable since they may cause difficulties in the separation of the treated fat from the reaction mixture. The extract of enzyme material may be termed an infusion in the sense that it may be prepared by steeping solid enzymic material in water or other fluid without boiling. It is believed that the desired effect in the present process is produced by an enzyme or a group or system of enzymes characterized by the ability thereof to effect the oxidation of more or less unsaturated fatty matter with formation of fat peroxide material as one of the reaction products or of fat peroxide material as the sole reaction product.

When employing extracts of fat-peroxidizing enzymic material, it has been found advantageous to have on hand a dilute extract and a concentrated extract, the preparation of which will be described below in detail. One general method that has been found particularly effective in the treatment of oils resistant to oxidation as well as oils that are readily oxidized, comprises mixing together a dilute enzyme extract and fatty

material in the presence of oxygen or a gas containing free oxygen and at the end of each of several successive periods of agitation adding a portion of concentrated enzyme extract.

A modification of the above method comprises adding concentrated enzyme extract at a predetermined rate to fatty material in admixture with water or a solution containing calcium ions while agitating in the presence of oxygen or gas containing free oxygen. Still another modification comprises agitating in the presence of oxygen or gas containing free oxygen a mixture of fatty matter and dilute enzyme extract or a solvent for enzymes and at the end of each of several successive periods of agitation placing solid material containing extractable enzymes in a zone in the mixture, the zone being bounded by a perforated medium penetrable to the liquid in the mixture but not to the solid material. In this instance an infusion of enzymic material is continuously formed in the reaction mass which offsets the depletion of the enzyme during the treatment.

By employing the above processes the enzymes are finely dispersed throughout the reaction mass. Better control of oxidation is thereby obtained. Also, the amount of enzymic material and the time of treatment necessary in conducting the reaction is lessened to an extraordinary degree. Less time is required even though no adjuvant such as hydrogen peroxide is employed, the addition of which is disclosed in the above-mentioned Patent No. 1,994,992.

It has been found that ordinarily there appears to be a serious impairment of enzyme activity during aeration. In order to reduce exposure of the enzymes to air, it is thought best not to add the active enzyme material all at one time, except in cases where oxidation is very rapid as for instance in the case of cottonseed oil. It is not definitely known whether the impairment is due to the destruction of enzymes due to oxidation, or whether the enzymes are adsorbed by the protein material and thus rendered inactive, but variations have been noted in the resistance of different oils to enzyme-oxidation. Refined peanut oil was found to be more resistant than cottonseed oil and refined English peanut oil was found to be somewhat more resistant than domestic refined peanut oil as obtained in the United States in obtaining the same degree of enzyme-oxidation. Sesame oil behaves about the same in this respect as peanut oil.

In regard to the relative proportions of fatty material and aqueous media mentioned above as a factor in the present process, it is noted that the presence of large quantities of water in the reaction mass is highly beneficial. The amount of water used varies with the particular fatty material under treatment and the emulsifying properties of the enzyme extracts or concentrates used. With substantially equal volumes of aqueous extract and oil in the reaction mass or where a greater amount of water is present than that of the oil, separation of the treated fatty product is relatively simpler.

The temperature at which the treatment of fatty material is carried on need not be higher than substantially 90° F. and may be as low as 32° F. or as high as 140° F. Temperatures around 70° F. to 78° F. are found desirable. Optimum results are achieved with temperatures ranging from about 70° F. to about 78° F. operating in conjunction with features hereinbefore recited.

The degree of oxidation of an oxidizable organic material prepared in the present process, is readily determined by quantitative methods and may be expressed in terms of molecules (M) of hydrogen peroxide per 1000 grams of organic material as indicated below, or in terms of grams of hydrogen peroxide per one hundred grams of organic material, an M-value of 0.100 being equivalent to 0.34 grams hydrogen peroxide per 100 grams of oil.

Extraction of Enzymic Agents

As indicated above, extracts of enzymic materials may be prepared for use in the above process and such extracts may be either dilute or concentrated. By way of example, a dilute extract may be prepared for use in the following example as follows:

7.6 parts by weight of active soya flour are stirred in about 730 parts by weight of a cold 0.1% calcium chloride solution in an aqueous medium. After thirty minutes of moderate agitation, the suspended matter is allowed to settle or the solution is centrifuged. The slightly turbid, supernatant solution may then be siphoned off. If desired, the solution may be filtered and run directly into a tank in which fatty material is to be treated.

A concentrated enzyme solution may be prepared as follows: About 15.7 parts by weight of active soya flour are stirred into 126 parts by weight of a 0.2% calcium chloride solution in an aqueous medium and gently agitated for about 30 minutes. The mixture is allowed to settle at a relatively low temperature preferably slightly below room temperature and the supernatant liquid may be siphoned off and used as needed.

Production of Enzyme Oxidized Products

Example I

About 70 parts by volume of English refined peanut oil and about 75 parts by volume of dilute enzyme extract prepared as set forth above are placed in a tank. The temperature of the oil and the extract is about 54° F. The mixture is agitated vigorously for about 15 minutes, at the end of which time 3 parts by volume of a concentrated extract, prepared as directed above, are added. Another 3 parts by volume of concentrate are added 15 minutes later, and two more additions of concentrate of substantially the same amount are made at 15-minute intervals. Thus after 60 minutes of agitation, about 12 parts by volume of enzyme concentrate may be added. After the last addition of concentrate, agitation is continued for about 38 minutes, the total time of agitation being about 98 minutes. The agitation is conducted in the presence of air or other inert gas containing free oxygen.

By running control tests, the M-value of the oil determined at different intervals of time is found to be as follows in the above procedure:

M-value after 15 min. reaction time	0.035
M-value after 30 min. reaction time	0.050
M-value after 45 min. reaction time	0.060
M-value after 60 min. reaction time	0.071
M-value after 98 min. reaction time	0.098

After the treatment outlined above, the reaction mixture is heated to about 110 to 115° F. to effect more perfect separation, and run through a centrifuge. A clear oil is obtained after running the mixture through the centrifuge just once. This oil is shown to have an M-value of 0.100.

Example II

A mixture of 2600 parts by volume of domestic refined peanut oil and 2288 parts by volume of dilute enzyme extract are mixed together. Instead of using the dilute enzyme extract mentioned above, the dilute extract, if desired, may be obtained by treating about 3 parts by weight of active soya flour with 300 parts by weight of a 0.15% solution of calcium chloride in an aqueous medium and agitating for 30 minutes, whereupon the mixture is allowed to settle and the supernatant liquid drawn off.

Before commencing the oxidation process, several portions of active soya flour (100% passing through 20 mesh and 100% retained on 40 mesh screen) each amounting to about 1% of the weight of oil treated, are each put in a small cloth bag and each dipped in about four times its weight of a dilute enzyme extract or an aqueous medium for about 25 to 66 minutes to thoroughly wet and soak the meal.

After the liquid mixture of oil and dilute enzyme extract is agitated for about 15 minutes, one of the bags referred to above is submerged in the mix so that it is covered completely by the liquid mixture and is in a zone of violent circulation of the mix. The agitation is continued for about 15 minutes, after which time a second bag of soaked meal is introduced in the same way as the first, and 15 minutes later the first bag is replaced by a third bag of soaked meal. The agitation is continued in the presence of air until the total time is about 90 minutes. The temperature may be about 65° F. at the start and may rise to about 76° F. at the end of the treatment. The progress of the treatment is shown in the following table at different time intervals:

M-value of oil after 15 min. treatment	0.033
M-value of oil after 30 min. treatment	0.052
M-value of oil after 45 min. treatment	0.069
M-value of oil after 60 min. treatment	0.087
M-value of oil after 75 min. treatment	0.092
M-value of oil after 90 min. treatment	0.100

The mixture resulting from the above treatment is run through a super-centrifuge once and a treated oil practically free of suspended matter and water, and of good odor and bland flavor is obtained.

Example III

About 235 parts by weight of domestic refined peanut oil and 260 parts by weight of cold water containing about 0.4 part by weight of calcium chloride are brought together in a mixing tank and a bag of soaked meal prepared as described above is immersed in the mix. After 30 minutes of agitation in the presence of air, the bag is replaced by a second bag and at the end of another 15 minutes of agitation in the presence of air, a third bag is introduced, both bags remaining in the mix until agitation is stopped. The agitation period in the presence of air may total 105 minutes. The temperature of the mix may vary from about 70° F. to about 77° F.

The progress of the treatment is illustrated by the following data:

M-value of oil after treatment of 15 min.	0.027
M-value of oil after treatment of 30 min.	0.036
M-value of oil after treatment of 45 min.	0.050
M-value of oil after treatment of 60 min.	0.068
M-value of oil after treatment of 75 min.	0.081
M-value of oil after treatment of 90 min.	0.087
M-value of oil after treatment of 105 min.	0.096

Example IV

About 2600 parts by volume of English refined peanut oil and about 2500 parts by volume of dilute enzyme extract are brought together and agitated in the presence of air. After 15 minutes of agitation, about 400 parts by volume of concentrated enzyme extract are permitted to flow into the mix at the rate of about 8.5 parts by volume per minute, requiring about 45 minutes to add the 400 parts. The temperature of the mix is maintained preferably around 70 to 75° F.

The progress of the above treatment is indicated by the following data:

M-value of oil after 15 min. agitation	0.031
M-value of oil after 30 min. agitation	0.052
M-value of oil after 45 min. agitation	0.069
M-value of oil after 60 min. agitation	0.085
M-value of oil after 75 min. agitation	0.098
M-value of oil after 80 min. agitation	0.102

Super-centrifuging of the mix resulting in the above procedure readily brings about separation of the oxidized oil and renders it practically free from suspended matter and moisture.

Example V

About 2600 parts by volume of English peanut oil and 2500 parts by volume of cold water are brought together and agitated. On starting the agitation about 100 parts by volume of enzyme concentrate are quickly added and the agitation in the presence of air is continued for a total time of about 70 minutes. During this period of agitation and after adding the above 100 parts of concentrate, an additional 400 parts by volume of concentrate are added at the rate of 100 parts by volume per 15 minutes. The temperature of the mix is about 66° F. at the start and may rise to about 78° F. in the course of the period of agitation.

The progress of the reaction is shown by the following:

M-value of oil after 15 min. treatment	0.032
M-value of oil after 30 min. treatment	0.056
M-value of oil after 45 min. treatment	0.075
M-value of oil after 60 min. treatment	0.093
M-value of oil after 70 min. treatment	0.099

The treated oil is separated from the mix preferably by supercentrifuging.

Example VI

About 70 parts of volume of refined cottonseed oil (salad oil) and about 75 parts by volume of dilute enzyme extract are brought together in a tank provided with an agitator. The dilute enzyme extract in this instance may be prepared from about 6.5 parts by weight of active soya flour suspended in about 609 parts by weight of 0.1% calcium chloride solution in an aqueous medium. The suspension is stirred for about 30 minutes and the mix then allowed to settle and the extract filtered through a 40 mesh strainer.

Agitation of the oil and the enzyme extract is continued in the presence of air for about 105 minutes, during which time the temperature is raised gradually from about 48° F. to about 70° F. The agitation is then interrupted for about 15 minutes during which period the mix is warmed up gradually to about 90° F. Agitation is then resumed at the latter temperature in the presence of air and the mix run through a supercentrifuge within a period of about 45 minutes.

The temperature of the mix and the progress

of the reaction are shown in the following tabulation:

M-value	Time of agitation	Temp. of mix
	Minutes	°F.
Nil	0	48
0.043	30	
0.069	60	63
0.083	90	68
0.089	105	70
0.095	120	90
0.098	135	90
0.103	150	90

The fatty product obtained in the process of the present invention is of superior quality, particularly for edible purposes. For instance, it may be employed in baking both as a shortening and as a bleaching agent. As a shortener, the oxidized fat serves to make a baked product more tender, and easy to break. As a bleaching agent for bread dough, for instance, the product of the present invention is mixed with bread-making ingredients as in Patent No. 1,994,993, either in place of the usual shortening agent or as a part of it. After baking the dough having therein the oxidized oil, it is noted that the baked product is whitened and has a good odor and taste, and a good texture as well.

It has been found that the type of emulsion formed by mixing together an oil or fat, enzyme extract or suspension, and air (oxygen) is greatly affected by (1) the ratio of the amounts of oil or fat, enzyme source, enzyme solvent, and gas (air or oxygen) present in a reaction mixture; (2) the nature of the enzyme source and the enzyme solvent; and (3) the mechanical means employed to agitate the reaction mixture.

The type of emulsion that is formed has been found to exert a marked influence on: (*a*) the viscosity of the reaction mixes; (*b*) the degree of aeration obtainable with given mechanical means; (*c*) the proper contacting of all phases concerned (liquid oil or fat, liquid aqueous phase, and gaseous-oxygen-carrying phase); (*d*) the efficiency of mechanical methods (such as supercentrifuging) for isolating the treated oil or fat from a reaction mixture; and (*e*) the degree of enzyme-oxidizability of the oil or fat treated.

Reaction mixtures in the form of emulsions having certain viscosities are in some cases quite stable. The oil or fat being mostly the interior phase of the emulsion, the large amounts of highly hydrated protective colloids primarily in the form of soluble proteins render it extremely difficult to isolate the oil or fat by the use of the most effective centrifugals available. High losses of oil and the application of excessive effort are unavoidable.

By the use of the present method, as illustrated in the examples given, reaction mixtures are prepared which have consistencies and viscosities very little different from those of oil and water mixtures. The reaction mixtures contain very small amounts of, for instance, not unduly hydrated proteins, which, under conditions stated are not adapted to act as protective hydrophillic colloids and, for this reason, permit of the isolation of the treated oil or fat free from suspended matter (proteins) and water in a single centrifuging operation without undue losses of material and with a marked saving of labor. Such qualities are characteristics of an unstable dispersion, which, in the present process is maintained during agitation of the fatty material and water in the peroxidation treatment.

Although fatty oils treated by the process of the present invention may be used by themselves in the bleaching of dough, it has been found to be advantageous to blend an enzyme oxidized oil such as enzyme oxidized peanut oil, cottonseed oil, corn oil, or other enzyme oxidized oil or fat with a hard fat or a fatty material that is solid at ordinary temperatures and that has not been treated by the present process. Sufficient of the latter fatty material is added to form with the treated oil or fatty material a solid or semi-solid blend having dough-bleaching properties.

For instance, enzyme oxidized peanut oil having an M-value of about 0.100 may be blended with about 15% to 18% of its weight of cottonseed oil stearin (60° titre) or hydrogenated whale oil. The hard fats are blended in the melted state and the resulting mixture including the enzyme oxidized oil is run over chill rolls in the usual way and homogenized by means of a texturator. The resulting product has the appearance of a solid fat and has an M-value of approximately 0.08. As little as 1% of the blend incorporated in a bread dough made from unbleached flour, produces a good bleaching and a good shortening effect.

As seen from the above, fatty products having both shortening and bleaching properties and having highly desirable physical characteristics such as bland flavor, good wholesome odor, and substantially free from water and suspended matter, may be efficiently and economically prepared.

By means of the process disclosed and claimed herein, an enzyme oxidized fatty material with a high M-value may be obtained at relatively low temperatures in less time with a minimum loss of fatty material and with a saving in enzymic material. Only about one to five pounds of soya flour, for instance, are needed for each 100 pounds of oil. Although higher temperatures than those disclosed herein may be employed to obtain an oxidized fatty product, the use of the lower temperatures and the prevention of the oil during treatment from contacting with the enzyme source from which the enzyme extracts are prepared, as pointed out, results in a product that is highly improved from the standpoint of color, taste and odor. It is thus rendered more readily salable.

We claim:

1. A process of preparing peroxidation products of fatty material having dough-bleaching properties, and containing substantially no water and suspended matter, which process comprises suspending in an emulsified mixture of liquid fatty material and water in substantially equal proportions peroxidizing enzyme-containing material, subjecting the said mixture to vigorous agitation and concurrently therewith introducing additional peroxidizing enzyme material and thereby maintaining an unstable dispersion of enzyme material in the mixture, and also concurrently therewith introducing gas containing free oxygen and maintaining the fatty material in liquid form, thereby effecting the catalytic oxidation of the fatty material to provide peroxidation of the latter, and subjecting the resulting treated unstable dispersion of fatty material and water to supercentrifugation which promoted by the substantially 1:1 mixture of liquid fatty material and water effects separation of a substantially water-free and suspension-free peroxidized fatty product from the said mix-

ture which product when used as an ingredient in the baking of bread imparts a bleaching effect without adversely affecting the flavor or odor.

2. A process of preparing peroxidation products of fatty material having dough-bleaching properties, and containing substantially no water and suspended matter, which process comprises suspending in an emulsified mixture of liquid fatty material and water in substantially equal proportions peroxidizing enzyme-containing material from which an infusion of peroxidizing enzyme material is continuously supplied to the said mixture, subjecting the said mixture to vigorous agitation and concurrently therewith introducing peroxidizing enzyme material and thereby maintaining an unstable dispersion of enzyme material in the mixture, and also concurrently therewith introducing gas containing free oxygen and maintaining the fatty material in liquid form and at intervals during the said agitation augmenting the supply of peroxidizing enzyme material while maintaining a substantially 1:1 ratio of fatty material and water, thereby effecting the catalytic oxidation of the fatty material to provide peroxidation of the latter, and subjecting the resulting treated mixture of fatty material and water to supercentrifugation which promoted by the substantially 1:1 mixture of liquid fatty material and water effects separation of a substantially water-free and suspension-free peroxidized fatty product from the said mixture, which product when used as an ingredient in the baking of bread imparts a bleaching effect without adversely affecting the flavor or odor.

3. A process of preparing peroxidation products of fatty material having dough-bleaching properties, which process comprises vigorously agitating a mixture containing substantially equal proportions of fatty material and water while passing gas containing free oxygen therethrough and concurrently with such agitation and passage of the said gas, adding to the mixture relatively small amounts of peroxidizing enzyme-containing solution concentrated to such an extent that the total amount of solution added during the peroxidation does not substantially change the 1:1 ratio of fatty material and aqueous phases and does not substantially increase the total volume of the reaction mixture, whereby objectionable emulsification phenomena are prevented, and separating from the resulting treated mixture a peroxidized fatty product which when used as an ingredient in the baking of bread imparts a bleaching effect without adversely affecting the flavor or odor.

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