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Gray

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[54] **STABILITY OF ENZYMES**

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[58] Field of Search.....**195/31, 62, 63, 66, 68; 99/217, 221; 424/101; 21/54**

[56]

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[57]

ABSTRACT

Enzymes in an aqueous medium are cooled to 50° F. or below and then subjected to microwave energy under controlled conditions whereby the stability of the enzymes against deterioration is materially increased.

20 Claims, No Drawings

STABILITY OF ENZYMES

BACKGROUND OF THE INVENTION

Enzymes are generally unstable on standing in liquid aqueous medium whether in their natural menstruum (after removal from the living cell source) or after isolation from their natural menstruum and resuspension or redissolution (reconstitution) in a prepared aqueous medium. Thus, commercial enzymes, that is enzymes prepared and marketed for industrial, including medical, use, are normally dried, although in some instances certain enzyme preparations can be marketed as concentrated syrups. Some deterioration of the enzymes can occur by the time complete drying or concentration can be achieved. For ultimate use, the dehydrated or concentrated enzymes are often reconstituted by suspension or solution in an aqueous medium. This reconstitution affords an opportunity for error particularly where the reconstituted material must have a definite activity value per unit weight or volume. In addition, the reconstituted enzyme is subject to deterioration, the rate and extent depending upon various factors, such as temperature, concentration, time and the nature of the reconstituting medium, such as pH.

For example, in automated electronic diagnostic procedures for diagnostic study of body fluids, like blood, blood plasma, blood serum and urine, a control blood serum is required which should be as close as possible to fresh, natural and normal blood serum. Blood serum, of course, contains many enzymes. In the past, it has been necessary to lyophilize blood serum marketed for this purpose. The technician then had to reconstitute the lyophilized material with water for use in his procedures. This presented limitations and disadvantages. Lyophilization itself can upset the delicate balance of constituents of the serum. Reconstitution of the lyophilized materials often resulted in serious errors. Often a plurality of different lyophilized blood serum products had to be prepared from which the technician had to select one or more depending upon the particular diagnosis he was to perform. Moreover, the reconstituted material had limited shelf life, so that a fresh package of lyophilized material normally had to be reconstituted and used daily.

The foregoing exemplifies problems encountered through instability of enzyme preparations, and has been discussed at length since blood serum represents a particularly delicate and unstable enzyme-containing material to which the present invention is particularly applicable. However, as will appear hereinafter, the present invention is applicable to enzymes in general, whether in their natural menstruum (after removal from their natural living cell source or environment) or reconstituted.

It is the principal object of the present invention to provide a method for improving the stability of enzymes.

It is another object of the present invention to provide a method for markedly enhancing the stability of enzymes against deterioration in aqueous media.

It is a specific object of the present invention to provide a method for improving the stability of the enzymes in blood serum.

Other objects, including the provision of a novel blood serum of improved stability, will become apparent from a consideration of the following specification and claims.

The method of the present invention comprises cooling an enzyme in aqueous medium to below 50° F.; and, in quick succession, (a) subjecting the enzyme in a treating zone, while held in a closed, microwave-permeable container within said treating zone, to microwave energy through a moving atmosphere of coolant gas, for a period of time short of inactivation of the enzyme, said moving atmosphere being in direct contact with microwave-permeable walls of said container but out of direct contact with said enzyme and being at a temperature below 60° F. upon its admission to said treating zone, (b) discontinuing exposure of said enzyme to said microwave energy at the end of said period, and (c) cooling said enzyme. Preferably, the circulation of coolant gas is continued, after

discontinuance exposure of the enzyme to the microwave energy, for a period to provide at least a portion of the stated cooling. Preferably also, the enzyme material is cooled to below 50° F., and at least the latter portion of this cooling may be achieved by other means, as by contacting a container of said enzyme material with a cold liquid, like cold water. It is also preferred that the container of enzyme-material be rotated during the exposure to the microwave energy at a rate to provide at least one complete rotation of 360° during said exposure, and preferably a plurality of 360° rotations, to insure presentation of all major walls of the container to the microwave energy during the stated exposure.

It has been found that the foregoing treatment is capable of markedly enhancing the stability of the treated enzymes against deterioration, that is, against permanent loss of activity. It is known that enzymes can become deactivated, even permanently, by more drastic thermal treatments, including exposure to microwave energy. The treatment of the present invention, through the effects of precooling and rapid post-cooling, in combination with the effects of the coolant gas during exposure to microwave radiation and the limited extent of the latter, prevents deactivation of the enzyme while at the same time enhancing the stability of the enzyme. This is illustrated in the examples set forth hereinafter, and especially so in the example dealing with blood serum.

Stabilization according to the present invention is accomplished rapidly—in a matter of seconds—as well as simply and economically. Conditions can be standardized for any particular enzyme or mixture of enzymes to provide reproducible results from time to time. Thus, each lot or batch can be assayed by techniques specific for each enzyme.

As far as is presently known, any enzyme should be susceptible to improvement in stability according to the present invention. Enzymes are proteins, including metalloproteins and conjugated proteins, and are produced by living cells. Although there are various classifications of enzymes, one accepted classification is according to Webb, *Biochemical Engineering*, D. Van Nostrand Co., Inc., Princeton, N.J., 1964 (see also *Encyclopedia of Chemical Technology*, Kirk-Othmar, Second Ed., Vol. 8), as follows: (1) hydrolyzing enzymes—proteases and peptidases, such as pepsin, rennin, and the like; carbohydrases, such as amylases; esterases, such as lipases, phosphatases, and the like; urease; deaminase; etc.; (2) transferring enzymes—dehydrogenases, like lactic dehydrogenase; oxidases; transaminases, like glutamic transaminases; kinases, like creatine phosphokinase; (3) addition and subtraction enzymes—like aconitase, enolase, carboxylase and aldolase; (4) isomerases—like alanine racemase; (5) synthetases—like glutamine synthetase; and (6) nucleases—like deoxyribonuclease. As stated, the present invention is particularly applicable to the treatment of enzymes in blood serum which include phosphatases, transaminases, dehydrogenases and phosphokinase. Any one or all of these may be isolated from blood serum and treated separately, after reconstitution, if necessary, or blood serum as such may be treated. Blood serum, which is the clear liquid remaining after removing cellular elements (red and white cells and platelets) and the coagulating mechanism (fibrinogen) from whole blood, is one of the particularly preferred materials treated in accordance with the present invention.

The enzyme treated in accordance with the present invention will be in an aqueous medium—that is, it will not be dry. It may be in its natural menstruum with or without concentration or it may be reconstituted by suspension or dissolution in a prepared aqueous medium after isolation from or concentration in its natural menstruum. The amount of aqueous medium associated with the enzyme is not critical, so long as sufficient is present to wet the enzyme, and may well be dictated by the ultimate use for the product or handling considerations. For example, it is often most convenient to work with a liquid suspension or solution.

As is well-known, microwave energy is the electromagnetic wave energy of the wave length falling in the microwave re-

gion of the electromagnetic spectrum. The Federal Communications Commission has presently set aside, for microwave processing, bands of microwave energy within the range of between about 400 and about 20,000 megacycles per second, with a wave length ranging from about 13 inches for the lower frequencies to about 0.7 inch for the highest frequencies; specifically frequencies of about 890-940 with a wavelength of about 13 inches; frequencies of about 2,400-2,500 with a wavelength of about 4-5 inches, and frequencies of 17,850-18,000 with a wavelength of about 0.7 inch. However, the presently preferred microwave energy for use according to the present invention is an intermediate range having a frequency of from about 1,000 to about 5,000, and more particularly from about 2,000 to about 3,000, megacycles per second. Microwave energy is generated from a suitable high frequency source, such as a magnetron.

One feature of the present invention is the precooling of the enzyme material. Thus, the material at the time it is first exposed to the microwave energy should be well below room temperature, that is below about 50° F. While it may actually be frozen, since it will thaw upon exposure to the microwave energy, there is no need for this and, for ease of handling, it is preferably at a temperature above freezing. A temperature in the range of from about 35° to about 45° F. is particularly satisfactory. The enzyme material may be precooled outside the treating chamber or zone or it may be precooled within the treating chamber or zone by preliminary flow of the coolant gas before generation of the microwave energy.

Another feature of the present invention is holding the enzyme material being treated in a sealed container during the treatment. The walls of the container may be conventional, substantially gas-impermeable packaging materials like glass, polymethylmethacrylate, polystyrene and polyethylene, as in bottles, flasks, and pouches. The container will be essentially gas tight.

The container holding the enzyme material during exposure will be held in a larger treating chamber or zone into which the microwave energy is directed to penetrate the microwave-permeable wall of the container and permeate the enzyme.

Still another feature of the invention is the circulation of a coolant gas through the treating chamber or zone and around the walls of the container holding the enzyme material. The coolant gas employed may be any substantially inert (nonreactive with the environment in the presence of microwave energy) gas existing as a gas at the temperatures employed, especially air, nitrogen or carbon dioxide. While gases like argon, helium, neon, krypton, xenon, ethylene oxide, and mixtures thereof, and the like, are equivalent, they are less desirable at the present because of their cost.

The temperature of the coolant gas entering the treating zone should be below about 60° F., and is preferably below about 55° F. While the temperature thereof may go as low as 0° F., there is no advantage in it going below about 20° F. and at such lower temperatures there may be freezing problems if an enzyme material is left in the treating zone containing the cold gas for extended periods after the source of microwave energy has been turned off. A temperature for the incoming gas between about 30° and about 50° F. has been found to be particularly suitable. The coolant gas will become warmed during its travel through the treating zone, particularly from contact with the walls of the container holding the enzyme material, and the warmed gas is removed from the treating zone making way for incoming coolant gas. When the gas is recirculated for reuse, the temperature thereof must be reduced back to the desired temperature for admission to the treating zone.

Since the principal function of the coolant gas is to keep the walls of the container at a temperature well below that of the enzyme material being treated, forcing the coolant gas into the treating chamber and past the walls of the container under at least some positive pressure (at least slightly above atmospheric pressure) provides more efficient overall cooling without some area or areas of the walls becoming insuffi-

ciently cooled. Pressures as low as 0.03 p.s.i.g. have been used and pressures as high as 50 p.s.i.g. may be desirable. Air is particularly satisfactory at low positive pressures, whereas a substantially oxygen-free gas, especially nitrogen, is preferred at higher pressures.

The precise time of treatment with microwave energy according to the present invention may depend somewhat upon the particular enzyme being treated, the volume of the enzyme material, the concentration of enzyme in the material being treated and the power of the microwave-generating means. In general, the time required is directly proportional to the volume of enzyme-containing material and concentration of enzyme therein and is inversely proportional to the power of the microwave-generating means. It has been found that the exposure time, in any case, will be at least about one second. It has also been found that overexposure results in complete inactivation of the enzyme. Since this is undesirable in accordance with the present invention the total exposure time will be short of that producing such complete inactivation. Since this time will differ, for reasons stated above, it may be necessary to run a preliminary test or tests to note the extent to which the particular enzyme undergoing treatment can be subjected to the microwave energy without becoming completely inactivated. Each enzyme has its own assay so that it can readily be determined whether or not a treated sample thereof has become completely inactivated. In any event, the time is short of that causing the temperature rise in the enzyme material to the temperature of permanent inactivation of the enzyme; generally the time is not longer than that causing a temperature rise in the enzyme material above about 125°-130° F., and in most cases the temperature rises to from about 100° to about 120° F. The enzyme material may be subjected, according to the present invention, to a single exposure or to a plurality of exposures to the microwave energy.

A feature of the preferred process of the present invention is the presentation of all the major walls of the container holding the enzyme material directly to the microwave energy during the stated exposure. This is most conveniently done, using an upright bottle as the container, by rotating the bottle about its longitudinal (vertical) axis at least once (360°), and preferably a plurality of times, during exposure of the bottle to the microwave energy directed toward the bottle in a generally horizontal direction, i.e., in a direction generally normal to the major walls of the container. This presents all major side walls of the container, whether it be circular, square, rectangular or polygonal in cross section, directly to the microwave radiation.

After exposure to the microwave energy for the required period of time, exposure to microwave energy is discontinued and the enzyme material is quickly cooled. It is preferred, in this regard, to continue the cooling effect of the coolant gas after exposure to the microwave energy has been discontinued in order to cool or chill the treated enzyme material, preferably down at least to about 85° F. This can be accomplished by leaving the container of enzyme material in the treating zone, through which is circulated the coolant gas, after the source of microwave energy has been turned off, or, in the case of a continuously moving line of containers of enzyme material, by extending the movement beyond the field of direct microwave exposure while continuing the flow of coolant gas in contact with the containers. On the other hand, the enzyme material may be cooled by other means, supplemental to or instead of, the foregoing means, as by contacting the walls of the container with a cold liquid, like cold water, or by placing the container in a refrigerator. In any event, it is preferable to cool the treated enzyme material to below 50° F.

The present invention will be more readily understood from a consideration of the following specific examples which are given for the purpose of illustration only and are not to be considered as limiting the scope of the invention in any way.

EXAMPLE 1

Lactic dehydrogenase ("LDH") was dissolved in a phosphate-buffered aqueous nonserum base (pH about 7.2) to provide 627 units/ml., and the solution was filtered through a bacterial filter. Small sterile glass bottles (7 ml. nominal capacity) were filled with the solution, capped and the bottles were cooled to 40° F. Forty-eight of the bottles were then placed in a pressure chamber equipped with a 2-kw. magnetron connected to a 220-volt source of alternating current and capable of delivering microwave energy into the chamber at about 2,450 megacycles per second. The bottles were divided into groups of three, and each group was held on a small individual turntable. All small individual turntables were held on a larger turntable, so that while the large turntable was rotating at 24 r.p.m., the small individual turntables were rotating at 60 r.p.m.; that is, the small individual turntables rotated 2.5 times for each revolution of the large turntable. The turntable assembly was made of polymethylmethacrylate. Cold nitrogen gas was flowed into, through and out of the chamber, at a pressure of 2.5 p.s.i.g., its inlet temperature being about 35° F. The magnetron was next turned on for 62 seconds, the magnetron and associated wave guide being positioned to direct the microwave energy in a horizontal direction toward the side walls of the bottles. The maximum temperature reached by the solution was about 120° F. After the magnetron was turned off (after the 62 seconds exposure), circulation of the cold nitrogen gas was continued for a short time until the solution had returned to about 80° F. Rotation of the turntable assembly was stopped, the chamber was opened and the bottles were removed from the chamber and immediately cooled to 40° F. in an icewater bath. The treated samples, along with untreated controls, were stored at various temperatures and assayed from time to time with the following results:

treated material:

40° F.—after 13 days, 583 units/ml.; after 20 days, 375 units/ml.

70° F.—after 13 days, 497 units/ml.; after 20 days, 375 units/ml.

100° F.—after 13 days, 520 units/ml.; after 20 days, 400 units/ml.

control:

40° F.—after 1 week, 315 units/ml.; after 13 days, 0.

70° F.—after 1 week, 0.

100° F.—after 48 hours, 0.

EXAMPLE 2

Serum glutamic oxalacetic transaminase ("SGOT") was dissolved in a phosphate-buffered aqueous nonserum base (pH about 7.2) to provide 37 units/ml., and filtered through a bacterial filter. The solution was bottled, treated, stored and assayed as in Example 1 with the following results:

treated material:

40° F.—after 13 days, 26.7 units/ml. after 20 days, 20 units/ml.

70° F.—after 13 days, 13 units/ml.; after 20 days, 10 units/ml.

100° F.—after 13 days, 16 units/ml.; after 20 days, 3 units/ml.

control:

40° F.—after 1 week, 0.

70° F.—after 4 days, 0.

100° F.—after 48 hours, 0.

EXAMPLE 3

Five milligrams of deoxyribonuclease, from bovine pancreas, were reconstituted with 1 ml. of distilled water and then diluted to 800 ml. with distilled water. The solution was filtered through a bacterial filter. The starting potency after filtration was 786 units/mg. Small sterile glass bottles (7 ml. nominal capacity) were filled with the solution, capped and cooled to 40° F. Various samples (24 bottles per lot) were then treated as in Example 1 but for different exposure times as follows:

Sample	Exposure time (seconds)	Peak temperature of sample
A	60	about 112° F.
B	68	about 115° F.
C	74	about 122° F.

Upon removal from the chamber at about 80° F. and cooling to 40° F. in an icewater bath, the treated samples, along with untreated controls, were stored at 77° F. for 21 days. All samples were then assayed by optical density in a Beckman DU spectrophotometer with ultraviolet range wavelength using the assay method according to Kunitz, M., *J. Gen. Physiol.*, 33, 349 (1950).

The results were as follows:

Sample	Assay* (units/mg.)
A	475
B	520
C	450
Control	200

*Averages of two bottles from each treatment, three separate samples from each bottle.

EXAMPLE 4

Liver fraction lactic dehydrogenase isoenzyme, extracted from bovine heart, was dissolved in sterile distilled water to 1 unit/ml., and the solution was filtered through a bacterial filter. Small sterile, glass bottles (7 ml. nominal capacity) were filled with the solution, capped and cooled to 40° F. Forty-eight of the bottles were then exposed to microwave energy in the presence of cold flowing nitrogen gas as in Example 1 but for an exposure time of 56 seconds. After removal from the chamber at a temperature of about 80° F., and cooling to 40° F., the treated samples, along with untreated controls, were stored at room temperature. The controls became completely inactive on standing overnight, whereas the treated material was as active as the original after standing for three weeks. At the end of three months, there was less than 10 percent loss in activity in the treated material.

EXAMPLE 5

Heart fraction lactic dehydrogenase isoenzyme, extracted from rabbit muscle, was dissolved in sterile distilled water to 1 unit/ml., and the solution was filtered through a bacterial filter. After bottling and capping, the material was treated and then stored as in Example 4. The controls became completely inactive on standing overnight, whereas the treated material was as active as the original after standing for three weeks. At the end of three months, there was less than 10 percent loss in activity in the treated materials.

EXAMPLE 6

An aqueous solution of amylase (1,000 units/ml.) was prepared and filtered through a bacterial filter, and 10 ml. portions thereof were placed in six small sterile glass bottles (10 ml. nominal capacity) which were then capped. The six bottles were then cooled to 40° F., and three of them were placed in a pressure chamber equipped with a 2-kw. magnetron connected to a 220-volt source of alternating current and capable of delivering microwave energy into the chamber at about 2,450 megacycles per second. Each bottle was held on a small individual turntable, each of which was held on a larger turntable, so while the large turntable was rotating the small individual turntables, and the bottles thereon, were also rotating. The turntable assembly was made of polymethylmethacrylate. The large turntable and the small individual turntables were rotated at 30 r.m.p. Cold nitrogen gas was then flowed into, through and out of the chamber, at a pressure in the chamber of 2.5 p.s.i.g., its inlet temperature being about 35° F. The magnetron was next turned on for 7 seconds, the magnetron and associated waveguide being positioned to direct the microwave energy in a horizontal direction toward the side walls of the bottles. After the magnetron was turned

off (after the 7 seconds exposure) circulation of the cold nitrogen was continued for a short time until the amylase solution in the exposed bottles had returned to about 80° F. The turntable assembly was stopped, and the bottles were removed from the chamber, immediately cooled to 40° F. in an icewater bath and, along with the three untreated control bottles, held at 40° F. for 48 hours. All samples were then assayed for amylase activity with the following results (figures are averages):

treated material	800 units/ml.
control	200 units/ml.

EXAMPLE 7

Fresh human blood serum was filtered through a bacterial filter and placed in sterile glass bottles, 30 ml. per bottle, and the bottles were capped and cooled to 40° F. Three of the bottles were then exposed to microwave energy in the presence of flowing nitrogen gas under pressure as in Example 6 but for 15 seconds. Upon removal from the chamber at about 72°-75° F., the bottles were immediately cooled to 40° F. in an icewater bath. The bottles, along with untreated controls, were then stored at 40° F., and assayed from time to time. By the end of two weeks, the biochemical assay values of the controls had changed so that they were no longer within the normal ranges for the enzymes. The treated material, upon continued storage at 40° F., was assayed, from time to time over a period of nine months with the following results:

TABLE 1

Constituent		Values after designated days					
		30 days	90 days	150 days	210 days	270 days	
Sodium	Upper limit.	145	143	142	142.5	142	
	Mean	141.8	140.8	140.9	141	141.5	
	Lower limit.	140	139	139.5	139.7	139	
Potassium	Upper limit.	4.3	4.4	4.3	4.4	4.4	
	Mean	4.0	4.0	4.0	4.3	4.3	
	Lower limit.	3.9	3.8	3.92	3.94	3.8	
Glucose	Upper limit.	101	100.4	102	100	100	
	Mean	98.8	99.0	98.8	97.5	97.5	
	Lower limit.	90.5	93.6	93.5	93.2	91.4	
Alkaline phosphatase	Upper limit.	34.1	34.5	35.0	34.0	34.1	
	Mean	32.9	33.2	33.4	33.0	33.0	
	Lower limit.	30.0	30.2	31.0	30.9	30.5	
SGOT*	Upper limit.	27.0	25.7	25.0	24.0	23.5	
	Mean	25.1	24.5	24.0	23.8	22.8	
	Lower limit.	22.5	21.8	21.0	20.0	20.0	
SGPT*	Upper limit.	21.0	20.7	20.0	20.0	19.0	
	Mean	20.2	19.0	19.2	19.0	18.7	
	Lower limit.	19.5	18.4	18.3	18.0	17.8	
LDH* (total)	Upper limit.	210	20.4	204	201	198	
	Mean	200	196	196	196	190	
	Lower limit.	190	192.2	192	190	188	
LDH fractionation isoenzymes liver fraction (LDH ₁)	Upper limit.	23.4	22.6	22.8	22.1	22.5	
	Mean	22.5	21.5	21.3	21.4	21.2	
	Lower limit.	21.2	20.4	20.0	19.4	19.0	
Heart fraction (LDH ₂)	Upper limit.	46.5	45.8	44.0	43.6	43.1	
	Mean	44.6	44.0	43.2	42.9	42.6	
	Lower limit.	40.8	41.8	41.0	40.2	40.3	
Remaining fractions (LDH _{2,3,4})	Upper limit.	134	134	134	132	132	
	Mean	132.5	132.4	132	131.4	130.2	
	Lower limit.	130.6	130.2	129.5	129.2	128	
Amylase	Upper limit.	68	67	65	66.5	65	
	Mean	65	64	63.6	63.7	62.6	
	Lower limit.	62.5	61.8	61.3	61.6	61.4	
Creatine phosphokinase (C.P.K.)	Upper limit.	6.1	5.8	5.7	(**)	(**)	
	Mean	5.3	5.4	5.0			
	Lower limit.	4.8	4.5	4.2			
Total protein	Upper limit.	7.5	7.5	7.5	7.5	7.4	
	Mean	7.2	7.4	7.3	7.3	7.3	
	Lower limit.	6.9	7.0	7.1	7.0	7.0	
Albumin	Upper limit.	4.00	4.25	4.12	4.19	4.11	
	Mean	3.82	3.87	3.90	3.87	3.92	
	Lower limit.	3.42	3.44	3.56	3.51	3.70	
Globulin	Upper limit.	3.00	3.02	3.00	2.94	2.98	
	Mean	2.80	2.90	2.88	2.91	2.90	
	Lower limit.	2.55	2.66	2.61	2.60	2.70	

*SGOT is serum glutamic oxalacetic transaminase; SGPT is serum glutamic pyruvic transaminase; LDH is lactic dehydrogenase.

**No further assays made.

Normal values for these constituents are as follows:

TABLE 2

Constituent	Normal values
Sodium	135-145 meq./l.
Potassium	3.5-5.0 meq./l.
Glucose	65-110 mg. %
Alkaline phosphatase	5-35 units (International)
SGOT	5-40 units (International)
SGPT	5-35 units (International)
LDH (total)	150-500 units (International)
LDH liver fraction	10-20% of total
LDH heart fraction	20-40% of total
LDH remaining fractions	35-55% of total
Amylase	50-160 units (Diatase)
Creatine phosphokinase	up to 35 units (International)
Total protein	6.8-8.0 gm. %
Albumin	3.25-5.70 gm. %
Globulin	1.5-3.0 gm. %

Considerable modification is possible in the enzyme materials treated as well as in the particular techniques employed without departing from the scope of the invention.

What is claimed is:

1. The method of improving the stability of an enzyme which comprises cooling an enzyme in aqueous medium to below 50° F., and then in quick succession, (a) subjecting the enzyme in a treating zone, while held in a closed, microwave-

The treated material showed normal electrophoresis throughout the testing period.

permeable container within said treating zone, to microwave energy having a frequency between about 400 and about

20,000 megacycles per second, through a moving atmosphere of coolant gas, for a period of at least 1 second but short of inactivation of the enzyme and of causing a temperature rise in the enzyme material above about 130° F., said moving atmosphere being in direct contact with microwave-permeable walls of said container but out of direct contact with said enzyme and being at a temperature below about 60° F., upon its admission to said treating zone; (b) discontinuing exposure of said enzyme to said microwave energy at the end of said period and (c) cooling said enzyme.

2. The method of claim 1 wherein said microwave energy has a frequency of from about 1,000 to about 5,000 megacycles per second.

3. The method of claim 2 wherein said microwave energy has a frequency of from about 2,000 to about 3,000 megacycles per second.

4. The method of claim 3 wherein said microwave energy has a frequency of from about 2,400 to about 2,500 megacycles per second.

5. The method of claim 1 wherein after exposure to the microwave energy the treated enzyme in the container is continued to be subjected to the action of said coolant gas.

6. The method of claim 3 wherein after exposure to the microwave energy the treated enzyme in the container is continued to be subjected to the action of said coolant gas.

7. The method of claim 1 wherein said enzyme in aqueous medium is at least one of those in blood serum.

8. The method of claim 1 wherein the enzyme in aqueous medium is blood serum.

9. The method of claim 8 wherein the blood serum is cooled to a temperature of from about 35° to about 45° F. before exposure to said microwave energy.

10. The method of claim 8 wherein the microwave energy has a frequency of from about 2,000 to about 3,000 megacycles per second.

11. The method of claim 8 wherein the blood serum does not exceed a temperature of about 125° F. during said exposure to said microwave energy.

12. The method of claim 8 wherein, after discontinuing the exposure of said blood serum to said microwave energy, said blood serum is cooled to below about 50° F.

13. The method of claim 9 wherein the microwave energy has a frequency of from about 2,000 to about 3,000 megacycles per second; wherein the blood serum does not exceed a temperature of about 125° F. during said exposure to said microwave energy; and wherein, after discontinuing the exposure of said blood serum to said microwave energy, said blood serum is cooled to below about 50° F.

14. The method of claim 1 wherein substantially all of the major microwave-permeable walls of said container are presented directly to said microwave energy during said exposure.

15. The method of claim 3 wherein substantially all of the major microwave-permeable walls of said container are presented directly to said microwave energy during said exposure.

16. The method of claim 5 wherein substantially all of the major microwave-permeable walls of said container are presented directly to said microwave energy during said exposure.

17. The method of claim 8 wherein substantially all of the major microwave-permeable walls of said container are presented directly to said microwave energy during said exposure.

18. The method of claim 10 wherein substantially all of the major microwave-permeable walls of said container are presented directly to said microwave energy during said exposure.

19. The method of claim 13 wherein substantially all of the major microwave-permeable walls of said container are presented directly to said microwave energy during said exposure.

20. Blood serum treated in accordance with the method of claim 8 and in which enzymes thereof possess improved stability against deterioration as compared to similar blood serum not so treated.

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