

PATENT SPECIFICATION

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(54) FERMENTATIVE PRODUCTION OF CITRIC ACID

(71) We, HITACHI CHEMICAL COMPANY LIMITED, a Japanese Company of No. 5-1, Marunouchi 1-chome, Chiyoda-ku, Tokyo, Japan, do hereby declare the invention, for which we pray that a Patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to the fermentative production of citric acid, particularly from purified oils having a low iodine value by the use of a yeast.

More particularly, the present invention relates to a process for the fermentative production of citric acid by the use of a citric acid-producing yeast belonging to the species *Candida lipolytica* or *Candida tropicalis*, wherein a purified low iodine value oil is used as the main carbon source in a culture medium, and a neutralizing agent is added thereto in specified amounts at specified times.

There has previously been proposed a process for the fermentative production of citric acid from a glucose by the use of *Aspergillus niger*. This process has, however, the disadvantages that the acid productivity of the strain is variable and maintenance of the strain is therefore troublesome, oxalic acid is formed as a by-product, a prolonged term of cultivation is required, and waste treatment is complicated.

Recently, as an alternative, there has been developed another process for the fermentative production of citric acid from n-paraffins available as petrochemical products by the use of a yeast. However, it is expected that the starting n-paraffins used in this prior process will be less available in view of problems concerning international petroleum resources and petrochemical industries.

Under these circumstances, a new fermentative process for the production of citric acid is desired wherein, a mass-producible, stable, readily available and less expensive raw material is utilized.

In order to discover a raw material which meets the aforesaid requirements, we first

attempted to employ an oil having a low iodine value, such as coconut oil or palm oil, as a raw material. However, it was found that such an oil disadvantageously separates from a culture medium and solidifies *in situ* at a temperature suitable for the citric acid fermentation, namely 30°C., due to its relatively high melting point. Thus, the solidified oil tends to adhere to the wall of a fermenter during cultivation or float off from the culture medium, and eventually cultivation with this material does not give a completely satisfactory result. On the other hand, it is known in the art to suitably effect cultivation by neutralizing with calcium carbonate in citric acid fermentation for accumulation of the acid and control of cultivation, but calcium carbonate tends to produce, in cooperation with the oil, a soap-like insoluble substance, which results in much easier separation of the oil from the culture medium and in turn provides an extremely unfavourable technical problem in cultivation procedures.

As a result of our further attempt to resolve the above problem and develop a new and more advantageous process for the fermentative production of citric acid, we found that the accumulation of a high concentration of citric acid in a high yield can be attained by the use of a purified low iodine value oil as a raw material under specific cultivation conditions, namely the specified amount and time of the neutralizing agent to be added in citric acid fermentation by the use of the specific yeast as noted above.

By means of our improvement, both oil separation and formation of soap-like insoluble substances during cultivation can be effectively avoided and, additionally, growth of the yeast and acid production can be remarkably promoted.

According to the present invention, there is provided a process for the fermentative production of citric acid, which comprises culturing a citric acid-producing yeast belonging to the species *Candida lipolytica* or *Candida tropicalis* in a culture medium employing therein as a main carbon source a purified low

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iodine value oil (as hereinafter defined), the said culture medium having, at the beginning of culturing, a neutralizing agent added thereto in an amount of from 0.05 to 0.1% by weight based on the culture medium, and additionally adding a neutralizing agent into the culture medium during the culturing so that the total amount, in weight %, of the neutralizing agent added in the culture medium is from 10 to 20% based on the weight %, based on the culture medium, of the said purified low iodine value oil.

In this specification, by a "purified low iodine value oil" there is meant a purified oil having an iodine value of not more than 80, a total content of moisture and impurity of not more than 0.2%, and an acid value of not more than 0.2. Representative examples of such oils are palm oil (an iodine value of 50), coconut oil (an iodine value of 10), palm kernel oil (an iodine value of 25), cacao butter (an iodine value of 33-40), Japan wax (an iodine value of 14-34), and mixtures thereof.

The purified low iodine value oil may be suitably employed in an amount of from 3 to 15% by weight based on the culture medium.

In the process of this invention, there may be employed any of the citric acid-producing yeasts belonging to the species *Candida lipolytica* or *Candida tropicalis*, but as representative examples thereof may be mentioned *Candida lipolytica* 281 and *Candida tropicalis*. These strains have been deposited as accession Nos. FERM-P No. 269 and FERM-P No. 266, respectively, with Technical Research Institute of Microbial Industry, Agency of Industrial Science & Technology, Ministry of International Trade and Industry, Japan and also as ATCC 20346 and 20345 with the American Type Culture Collection in Washington D.C., U.S.A.

As closely related prior art, it is disclosed in the Journal of the Agricultural Chemical Society of Japan, Vol. 43, No. 3, 154 (1969)

that citric acid may be produced from oils by the use of a yeast, *Candida lipolytica*. However, it is to be noted that in this prior art process a comparatively high yield of citric acid is obtained solely when a high iodine value oil such as linseed oil, soybean oil or rape oil is employed, whereas a low iodine value oil such as palm oil gives an extremely low yield (about 5%) of citric acid. On the other hand, those high iodine value oils used in the prior art process are now expensive and there is no likelihood of the price being reduced in near future, as compared with ease of availability and lower price of the oils employed in the process of this invention.

In order to more clearly demonstrate the difference between the present process and the above mentioned prior process, with respect to the yield of citric acid, some comparative tests are given below.

Experiment 1.

Comparison test between the present process and the prior art process.

A culture medium (1 liter) was prepared by employing the indicated oil (5% by weight), ammonium sulfate (0.2% by weight), C.S.L. (Corn Steep Liquor) (0.26% by weight), potassium phosphate (0.0125% by weight), magnesium sulfate (0.01% by weight as the heptahydrate), vitamin B₁ (100γ) and calcium carbonate (0.5% by weight), and making them up into a 1 liter solution with water (the weight % is based on the culture medium, i.e. W/V%). Each 50 ml. portion of the medium was poured into a 500 ml.-volume Sakaguchi flask and, after sterilization in the usual manner, one loopful of *Candida lipolytica* 281 was inoculated therein. Reciprocating-shaking of the culture was effected for 6 days at 30°C. and 140 r.p.m. The calcium carbonate was added in portions. The results are summarized in Table 1 below.

TABLE 1

Oil	Iodine value	Reference	Yield of citric acid (%)
			Present Process
Linseed	180	42	107
Soybean	133	53	102
Rape	100	13	—
Palm	50	5	155
Palm Kernel	25	—	120
Coconut	10	—	100

Experiment 2.

Effect of the amount of CaCO_3 added at the beginning of cultivation on the yield of citric acid.

The test was effected by repeating the

same procedure as in Experiment 1 except that 0.175% by weight of ammonium sulfate and the indicated CaCO_3 and oil were used.

The results are summarized in Table 2 below.

TABLE 2

CaCO_3 (% by weight) at the beginning of cultivation	Yield of citric acid (%)		
	Coconut oil	Palm oil	Palm kernel oil
0	44.7	90.2	53.9
0.02	64.6	113.5	78.8
0.05	97.0	156.2	120.3
0.1	99.5	155.0	116.6
0.2	70.7	152.8	74.0
0.5	6.9	110.3	20.6
0.7	(—)*	57.2	(—)*

In the above Table, in cases where the indicated amounts of CaCO_3 were 0—0.2%, the remaining calcium carbonate was added in portions or all at once 1 to 3 days after the initiation of the cultivation so that the total amount of CaCO_3 was 0.5% by weight. No addition of CaCO_3 was made in cases where the indicated amounts of CaCO_3 were 0.5 and 0.7%. The symbol (—)* indicates remarkable separation of the oil without any growth of the strain.

Experiment 3.

Effect of the amount of CaCO_3 on the yield of citric acid.

Following the same procedure as in Experiment 1 except that the indicated amounts of oil, ammonium sulfate and calcium carbonate were employed and cultivation was effected for 7 days with a seed culture (8% by volume) of *Candida lipolytica* 281, the results summarized in Table 3 below were obtained.

TABLE 3

CaCO ₃ (% by weight)	Yield of citric acid (%)		
	Coconut oil (5% w)	Palm kernel oil (5% w)	Palm oil (7% w)
0	0.5	1.5	3.0
0.1	30.3	20.1	25.3
0.2	51.6	55.4	45.6
0.5	99.5	109.2	97.5
0.7	98.0	116.0	112.0
1.0	72.7	96.6	148.3
1.2	66.1	91.8	138.6
1.5	56.7	74.0	136.0
2.0	58.4	73.8	115.3

5 In the above Table 3, 0.1% by weight of the calcium carbonate was added at the beginning of cultivation and thereafter the remainder was added in portions or all at once during a cultivation period of 1 to 4 days to give the indicated amount of CaCO₃ therein.

10 As explained hereinabove, it is essential for the fermentative production of citric acid according to this invention to control the amount of the neutralizing agent to be added and the time when the agent should be added, for the prevention of oil separation and formation of soap-like insoluble substances during the cultivation as well as the promotion of the growth of a strain and the production of the acid.

20 More specifically, the amount of the neutralizing agent to be added at the beginning of cultivation or culturing should be minimum required for the growth of a strain and the production of an acid, namely from 0.05 to 0.1% by weight based on the medium and the total amount of the neutralizing agent to be added should be from 10 to 20% by weight, based on the concentration (% by weight) of substrate or purified low iodine value oil. After the beginning of cultivation, the amount of the neutralizing agent to be added and the time of addition are not critical, but it is usual and preferable to add the specific amount of the neutralizing agent at the beginning of cultivation, a half amount of the remainder immediately when a strain grows and the pH

of the culture medium falls below 4.0 and the remaining half amount after 1 or 2 days. In this case, cultivation may be more satisfactorily effected by the addition into a culture medium from 0.05 to 0.1% by weight of carbon compounds well assimilable to the strain, for example glucose, glycerol, and oleic acid. Cultivation may also be favourably effected by the use of the seed yeast which is cultivated on a culture medium containing a substrate oil.

Neutralizing agents which may be suitably employed in the process of this invention are calcium carbonate, calcium hydroxide and mixtures thereof.

The liquid medium which may be employed in the process of this invention may contain a carbon source, a nitrogen source, an inorganic salt, and an organic nutrient source. It is to be noted that the carbon source should contain at least one purified low iodine value oil (as hereinbefore defined). The oils may be usually added to a culture medium all at once, portionwise or stepwise.

The nitrogen source may be suitably an organic nitrogen source such as yeast extract, bouillon, peptone, dry yeast, corn steep liquor (C.S.L.), defatted soybean hydrolysate, or urea, or an inorganic nitrogen compound such as ammonium sulfate or ammonium nitrate.

In some cases where vitamin B₁ is deficient in the organic nitrogen source, a suitable amount thereof may be additionally added to a culture medium, since the strain usually

requires an appropriate amount thereof for growing.

The inorganic salt which may be added into the culture medium is suitably magnesium sulfate or zinc sulfate.

In the process of this invention, it is advantageous to limit the concentration of phosphate in the culture medium so that the growth of a strain may be prevented to some extent for the production of a large amount of citric acid in the cultured broth. When the phosphate is excessively present in the culture medium, the yield of citric acid may be lowered due to the promoted growth of a strain.

Cultivation can be effected under aerobic conditions commonly utilized in shaking culture or cultivation under aeration and agitation. The cultivation temperature may be suitably about 30°C., preferably 30±3°C. The pH range during cultivation may be suitably adjusted to pH 2—7 and, particularly when acid production is highly active, the pH may be suitably 2—3, which leads to the advantage that saprophytic contamination can be effectively prevented. As fully described above, the neutralizing agent, e.g. calcium carbonate, may be used in order to neutralize the acid formed, but the amount of the agent to be used and the time of addition thereof should be controlled. Cultivation may be suitably completed in about 3 to 8 days. Other additives commonly used in the art, e.g. antifoaming agents and surfactants may be added to the culture medium, if necessary.

After completion of the cultivation, recovery of citric acid may be effected by procedures wherein mycelium is firstly removed from the cultured broth. In the prior art, a large amount of the neutralizing agent, e.g. calcium carbonate, was required for the accumulation of citric acid and therefore citric acid accumulated was in the form of insoluble calcium citrate. In the process of this invention, cultivation is effected within a lower pH range by limiting the amount of neutralizing agent added to be as small as possible so that the sole solid content in the culture medium is mycelium. Then, the mycelium is filtered off after cultivation, the neutralizing agent, e.g. calcium carbonate or calcium hydroxide, is added to the clear filtrate to form calcium citrate, the citrate is recovered by filtration and decomposed by the addition of a suitable acid such as sulfuric acid, and, after filtration, the filtrate is treated with active charcoal or an ion exchange resin and concentrated to yield crystals of citric acid which are then filtered and dried to give the desired citric acid.

The invention will be further described with reference to the following illustrative Examples.

Example 1.

A culture of *Candida lipolytica* 281 (ATCC 20346) was inoculated into 50 ml. of a sterilized aqueous medium containing purified palm oil (5%), ammonium sulfate (0.2%), C.S.L. (corn steep liquor) (0.26%), monopotassium phosphate (0.0125%), magnesium sulfate (0.01% as the heptahydrate), zinc sulfate (0.001%), vitamin B₁ (100 µg./liter), and calcium carbonate (0.1%) and was incubated in a 500 ml.-Sakaguchi flask on a reciprocating shaker of 140—160 rpm at 30°C. Immediately after the strain grew and the pH of the medium fell below 4.0, 0.2% by weight of calcium carbonate separately sterilized was aseptically added and, 1 or 2 days thereafter, 0.2% by weight of the carbonate was additionally added so that the total amount of calcium carbonate added was 0.5% by weight. Thus the neutralizing agent (calcium carbonate) was used in a total amount of 10% by weight of the purified low iodine value oil (purified palm oil). After cultivation for 6 days, the cultured broth contained 77.2g/liter of citric acid and 1.8g/liter of isocitric acid. Mycelia were removed from the cultured broth by centrifugation, and to the filtrate was added calcium carbonate to form calcium citrate, which was then decomposed with sulfuric acid, treated with active charcoal, and subsequently passed through a column of a cation exchange resin to yield a clear filtrate. The filtrate thus obtained was concentrated and cooled to crystallize citric acid. In this example, 73.2g. of citric acid was obtained per litre of the above cultured broth.

Example 2.

5% by volume of the seed culture of *Candida tropicalis* (ATCC 20345), which had been previously cultured in a culture medium containing coconut oil, was inoculated into 50 ml. of a sterilized aqueous medium containing purified coconut oil (6%), ammonium sulfate (0.175%), C.S.L. (0.2%), monopotassium phosphate (0.025%), vitamin B₁ (50 µg./liter) glycerol (0.1%), and calcium carbonate (0.05%) and was incubated in a 500 ml.-Sakaguchi flask on a reciprocating shaker of 140 r.p.m. at 28°C. In the same manner as in Example 1, portions of calcium carbonate (three portions in this Example) were separately added to the medium so that the total amount of calcium carbonate added was 0.65% by weight. Thus the neutralizing agent (calcium carbonate) was used in a total amount of 10.8% by weight of the purified low iodine value oil (purified coconut oil). After cultivation for 5 days, the cultured broth contained 56.3g/liter of citric acid and 1.5g/liter of isocitric acid. The cultured broth thus obtained was

treated in the same manner as in Example 1 to give 53.5g of citric acid per litre of the cultured broth.

Example 3.

5 A culture of *Candida lipolytica* 281 (ATCC 20346) was inoculated into 50 ml. of a sterilized aqueous medium containing purified palm kernel oil (7%), ammonium sulfate (0.25%), C.S.L. (0.3%), mono-
10 potassium phosphate (0.025%), vitamin B₁ (100 µg./liter), magnesium sulfate (0.01% a heptahydrate), oleic acid (0.05%) and calcium hydroxide (0.1%) and was incubated in a 500 ml. Sakaguchi flask on a reciprocating shaker of 120 rpm at 28°C. In the
15 same manner as in Example 1, two portions of calcium hydroxide were added, i.e. a first portion of 0.4% by weight and a second portion of 0.5% by weight were employed to provide a total amount of calcium hydroxide of 1.0% by weight. Thus the neutralizing agent (calcium hydroxide) was used in a total
20 amount of 14.3% by weight of the purified low iodine value oil (purified palm kernel oil). After cultivation for 7 days, the cultured broth contained 84.0g/liter of citric acid and 2.2g/liter of isocitric acid. The cultured broth thus obtained was treated in the same manner
25 as in Example 1 to give 79g of crystalline citric acid per litre of the cultured broth.

Example 4.

10% by volume of the seed culture of *Candida lipolytica* 281 (ATCC 20346) was inoculated into 20 liters of a sterilized aqueous medium containing purified palm oil (10%),
35 ammonium sulfate (0.3%), C.S.L. (0.26%), monopotassium phosphate (0.025%) vitamin B₁ (50 µg./liter, antifoaming agent (0.05%) and calcium carbonate (0.2%) and incubated in a 30-liter jar fermentor, with aeration of 3.5 liters/min., agitation of 420 rpm., an inner pressure of 0.1 kg/cm², and at 30±2°C. Immediately the strain grew and pH of the culture medium fell below 4.0, 0.3% by
40 weight of calcium carbonate separately sterilized was added and then two 0.5% by weight portions of calcium carbonate were added every other day to provide a total amount of calcium carbonate of 1.5% by weight. Thus the neutralizing agent (calcium carbonate) was used in a total amount of 15% by weight of the purified low iodine value oil (purified palm oil). Cultivation was completed in 102 hours and the cultured broth contained 150.5g/liter of citric acid and 2.7g/liter of isocitric acid. The cultured broth thus obtained was treated in the same manner as in Example 1 to give 139 g. of crystalline citric acid per liter of the cultured
60 broth.

The morphological details of the organisms used in the above Examples are as follows:

Candida lipolytica 281 (ATCC 20346)

(1) Growth in glucose-yeast extract-malt water:

At 25°C., after three days, true mycelia are formed. Cells are round, ovoid to long oval or ellipsoid (5 to 7 × 10 to 12µ), almost alone. The true mycelia are long.

At 17°C., but after one month, thick and wrinkled or smooth, and hard but fragile ring is formed. There is also produced sediment.

(2) Growth on glucose-yeast extract-malt extract agar:

At 25°C., after ten days, a colony rises as a whole, and is wrinkled or reticulate. Edge portion is ciliated. Cells are ovoid to ellipsoid (3 to 4.5 × 7.5 to 10µ), and almost alone.

(3) Slant culture:

At 17°C., after one month, loosely wrinkled, lustless, a little viscous. Surface is covered with white and short pseudo mycelia. Colour is light grey to yellowish white.

(4) Potato dextrose agar:

True mycelia are long and grow well. Short pseudo mycelia are also observed. Blastospores are ovoid to long oval.

Candida tropicalis (ATCC 20345)

(1) Growth in glucose-yeast extract-malt extract water:

At 25°C., after three days, cells are short oval to oval, occasionally spherical (4 to 8 × 5 to 11µ). Sediment and ring are formed.

(2) Growth on glucose-yeast extract-malt extract agar:

At 25°C., after one month, white to creamy, lustless and soft. Surface is smooth or partially striped and reticulate or wrinkled.

(3) Potato dextrose agar:

Pseudo mycelia are formed, which are long extended diverged pseudo phylae having blastoconidia. Verticils of blastospores are diverged or single chain. True mycelia are sometimes observed.

WHAT WE CLAIM IS:—

1. A process for the fermentative production of citric acid, which comprises culturing a citric acid-producing yeast belonging to the species *Candida lipolytica* or *Candida tropicalis* in a culture medium employing therein as a main carbon source a purified low iodine value oil (as hereinbefore defined), the said culture medium having, at the beginning of culturing a neutralizing agent added thereto in an amount of from 0.05 to 0.1% by weight based on the weight of the said culture medium, and additionally adding a neutralizing agent into the culture medium during the culturing so that the total amount, in weight %, of the neutralizing agent added to the culture medium is from 10 to 20% based on the weight %, based on the culture

treated in the same manner as in Example 1 to give 53.5g of citric acid per litre of the cultured broth.

Example 3.

5 A culture of *Candida lipolytica* 281 (ATCC 20346) was inoculated into 50 ml. of a sterilized aqueous medium containing purified palm kernel oil (7%), ammonium sulfate (0.25%), C.S.L. (0.3%), mono-
10 potassium phosphate (0.025%), vitamin B₁ (100 µg./liter), magnesium sulfate (0.01% a heptahydrate), oleic acid (0.05%) and calcium hydroxide (0.1%) and was incubated in a 500 ml. Sakaguchi flask on a reciprocating shaker of 120 rpm at 28°C. In the
15 same manner as in Example 1, two portions of calcium hydroxide were added, i.e. a first portion of 0.4% by weight and a second portion of 0.5% by weight were employed to provide a total amount of calcium hydroxide of 1.0% by weight. Thus the neutralizing agent (calcium hydroxide) was used in a total
20 amount of 14.3% by weight of the purified low iodine value oil (purified palm kernel oil). After cultivation for 7 days, the cultured broth contained 84.0g/liter of citric acid and 2.2g/liter of isocitric acid. The cultured broth thus obtained was treated in the same manner
25 as in Example 1 to give 79g of crystalline citric acid per litre of the cultured broth.
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Example 4.

10% by volume of the seed culture of *Candida lipolytica* 281 (ATCC 20346) was inoculated into 20 liters of a sterilized aqueous medium containing purified palm oil (10%), ammonium sulfate (0.3%), C.S.L. (0.26%), monopotassium phosphate (0.025%) vitamin B₁ (50 µg./liter, antifoaming agent (0.05%) and calcium carbonate (0.2%) and incubated
35 in a 30-liter jar fermentor, with aeration of 3.5 liters/min., agitation of 420 rpm., an inner pressure of 0.1 kg/cm², and at 30±2°C. Immediately the strain grew and pH of the culture medium fell below 4.0, 0.3% by weight of calcium carbonate separately sterilized was added and then two 0.5% by weight portions of calcium carbonate were added
40 every other day to provide a total amount of calcium carbonate of 1.5% by weight. Thus the neutralizing agent (calcium carbonate) was used in a total amount of 15% by weight of the purified low iodine value oil (purified palm oil). Cultivation was completed in 102 hours and the cultured broth contained 150.5g/liter of citric acid and 2.7g/liter of isocitric acid. The cultured
45 broth thus obtained was treated in the same manner as in Example 1 to give 139 g. of crystalline citric acid per liter of the cultured broth.
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The morphological details of the organisms used in the above Examples are as follows:

Candida lipolytica 281 (ATCC 20346)

(1) Growth in glucose-yeast extract-malt water:

At 25°C., after three days, true mycelia are formed. Cells are round, ovoid to long oval or ellipsoid (5 to 7 × 10 to 12µ), almost alone. The true mycelia are long.

At 17°C., but after one month, thick and wrinkled or smooth, and hard but fragile ring is formed. There is also produced sediment.

(2) Growth on glucose-yeast extract-malt extract agar:

At 25°C., after ten days, a colony rises as a whole, and is wrinkled or reticulate. Edge portion is ciliated. Cells are ovoid to ellipsoid (3 to 4.5 × 7.5 to 10µ), and almost alone.

(3) Slant culture:

At 17°C., after one month, loosely wrinkled, lustless, a little viscous. Surface is covered with white and short pseudo mycelia. Colour is light grey to yellowish white.

(4) Potato dextrose agar:

True mycelia are long and grow well. Short pseudo mycelia are also observed. Blast-spores are ovoid to long oval.

Candida tropicalis (ATCC 20345)

(1) Growth in glucose-yeast extract-malt extract water:

At 25°C., after three days, cells are short oval to oval, occasionally spherical (4 to 8 × 5 to 11µ). Sediment and ring are formed.

(2) Growth on glucose-yeast extract-malt extract agar:

At 25°C., after one month, white to creamy, lustless and soft. Surface is smooth or partially striped and reticulate or wrinkled.

(3) Potato dextrose agar:

Pseudo mycelia are formed, which are long extended diverged pseudo phylae having blastoconidia. Verticils of blasto-spores are diverged or single chain. True mycelia are sometimes observed.

WHAT WE CLAIM IS:—

1. A process for the fermentative production of citric acid, which comprises culturing a citric acid-producing yeast belonging to the species *Candida lipolytica* or *Candida tropicalis* in a culture medium employing therein as a main carbon source a purified low iodine value oil (as hereinbefore defined), the said culture medium having, at the beginning of culturing a neutralizing agent added thereto in an amount of from 0.05 to 0.1% by weight based on the weight of the said culture medium, and additionally adding a neutralizing agent into the culture medium during the culturing so that the total amount, in weight %, of the neutralizing agent added to the culture medium is from 10 to 20% based on the weight %, based on the culture



- medium, of the said purified low iodine value oil.
2. A process as claimed in claim 1, wherein the said purified low iodine value oil is palm oil, coconut oil, palm kernel oil, cacao butter, Japan wax or a mixture thereof.
3. A process as claimed in claim 1 or 2, wherein the said neutralizing agent is calcium carbonate, calcium hydroxide or a mixture thereof.
4. A process as claimed in any of claims 1 to 3, wherein the additional addition of the neutralizing agent is effected after the pH of the culture medium falls below 4.0.
5. A process as claimed in claim 4, wherein a half amount of the neutralizing agent additionally to be added is added immediately when a strain grows and the pH of the culture medium falls below 4.0 and wherein the remaining half amount is added after 1 or 2 days.
6. A process as claimed in any of claims 1 to 5, wherein the culturing is conducted at a temperature of $30 \pm 3^\circ\text{C}$.
7. A process as claimed in any of claims 1 to 6 wherein the culturing is conducted at a pH value of from 2 to 7.
8. A process as claimed in any of claims 1 to 7, wherein the purified low iodine value oil is employed in an amount of from 3 to 15% by weight based on the culture medium.
9. A process for the fermentative production of citric acid substantially as herein described in any of the foregoing Examples.
10. Citric acid obtained by the process as claimed in any of claims 1 to 9.
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* C L N S *