

OFFICIAL NORM MEXICAN NOM-119-SSA1-1994, Goods and services. RAW MATERIALS FOR FOODS, PRODUCTS OF PERFUMERÍA And BEAUTY. NATURAL ORGANIC COLORANTES. SANITARY SPECIFICATIONS.

To the margin a seal with the National Shield, that says: The Mexican United States. - Secretariat of Health.

JOSE MELJEM MOCTEZUMA, Chief of a main directorate of Sanitary Control of Goods and services, by agreement of the National Consultative Committee of Normalization of Regulation and Sanitary Promotion, with foundation in articles 39 of the Statutory law of the Federal Public Administration; 38. fraction II, 47 of the Federal Law on Metrología and Normalizacio'n; 194 fraction I of the General Law of Health; ò. fraction III, 659, 1238, 1243 and other applicable ones of the Regulation of the General Law of Health in the matter of Sanitary Control of Activities, Establishments, Products and Services; 8o. 13 fraction IV and fraction I of the Rules of procedure of the Secretariat of Health.

PREFACE

In the elaboration of the present norm to the following Organisms and Institutions participated:

HEALTH SECRETARY

National laboratory of Public Health
Main directorate of Sanitary Control of Goods and services

SECRETARY OF COMMERCE And INDUSTRIAL PROMOTION

Main directorate of Commercial Policies

FEDERAL PROCURADURIA OF THE CONSUMER

NATIONAL INSTITUTE POLITECNICO

National school of Biological Sciences

NATIONAL UNIVERSITY AUTONOMA OF MEXICO

Faculty of Chemistry

METROPOLITAN UNIVERSITY AUTONOMA

NATIONAL ASSOCIATION OF PRODUCT MANUFACTURERS AROMATICOS

NATIONAL CAMARA OF THE INDUSTRY OF THE TRANSFORMATION

NATIONAL CAMARA OF The INDUSTRY OF PERFUMERIA And COSMETICA

C.V COCA COLA DE MEXICO, S.A..

CONFEDERATION OF INDUSTRIAL CAMARAS

C.V INDUSTRIAS CUAMEX, S.A..

LABORATORIOS MIXIM, S.A..

C.V MANE DE MEXICO, S.A..

PROBAMEX, S.A. OF C.V.

C.V PRODUCTOS ROCHE, S.A..

SPECTRUM, S.A. OF C.V.

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0. Introduction

The dispositions of the present Mexican Official Norm are of public order and social interest and establishes the specifications of identity and purity of the natural organic colorantes; for its application in foods and products of perfumería and beauty, in which they are used like raw materials. When considering itself these like additives, its use can represent a risk for the health when being in them high levels of polluting agents, reliable substances or that are used for the extraction and which they are detrimental for the health from the consumer.

These specifications are only satisfied when in their elaboration raw materials of sanitary quality are used, are applied good practices of manufacture, are made in the premises and facilities under hygienic conditions, that assure that they are apt for use and human consumption, in agreement with the established thing by the General Law of Health, its Regulation and other applicable dispositions of the Secretariat of Health.

1. Objective and field of application

1.1 This Mexican Official Norm establishes the sanitary specifications that must fulfill organic the colorantes natural.

1.2 This Mexican Official Norm is of obligatory observance in the National Territory for the physical or moral people who dedicate to their process or import.

2. References

This norm is complemented with the following thing:

Synthetic organic NOM-038-SSA1-1993 Colorantes. General sanitary specifications.

NOM-116-SSA1-1994 food humidity Determination by heat treatment. Method by sand or Gauze.
*

NOM-117-SSA1-1994 Method of test for the determination of cadmium, arsenic, lead, tin, receives, iron, zinc and mercury in foods, potable water and water purified by spectrometry of atomic absorption. *

NOM-118-SSA1-1994 Raw materials for foods, products of perfumería and beauty. Colorantes inorganic. Sanitary specifications. *

3. Definitions

For the aims of this norm it is understood by:

3,1 Additives for foods, those substances that directly add to foods and drinks, during their elaboration to provide or to intensify aroma, color or flavor; in order to improve its stability or for its conservation.

3.2 Good practices of manufacture, set of norms and related activities to each other, destined to guarantee that the products have and maintain the specifications required for their use.

3,3 Colorante, material that color to another material distributes or mixes elaborated by a process of synthesis or similar; by extraction or separation, obtained of a source animal, vegetable or mineral and that later have put to the test themselves fehacientes of security that somewhere release for their product and food use of perfumería and beauty to it or of them and who directly or through its reaction with other substances is able to distribute the color that characterizes to him.

3,4 Natural organic Colorante, pigment or substance that obtain from vegetal matter or animal, with a limited chemical process or without him and put to the test later identity and purity that allows them to be used in foods, products of perfumería and beauty, somewhere of these or in everything and that directly or through its reaction with other substances, are able to distribute the color that characterizes to him.

3.5 Package, all container destined to contain a product and that makes contact with enemy with the same one, conserving its physical, chemical and sanitary integrity.

3.6 Secondary package, that that contains to the primary one.

3.7 Label, all label, label, inscription, image either another descriptive or graphical form or that is printed, noticeable, engraving, in relief, hollow, estarcido or adhered to the packing or package of the product.

3.8 Heavy metal or nonmetal, those chemical elements that even cause undesirable effects in the metabolism in low concentrations. Its toxicity depends on the doses in which they interfere, as well as on their accumulation in the organism.

3.9 Process, the set of activities relative to the obtaining, elaboration, manufacture, preparation, conservation, mixed, preparation, packaging, manipulation, transport, distribution, storage and expendio or provision to the product public.

4. Symbols and abbreviations

When in this norm reference to the following symbols and abbreviations becomes, it is understood by:

to alpha

b beta

cm centimeter

And extinction coefficient

CI Index Color

G gamma

g grams

°C Celsius degrees

kg kilogram

KPa Pascal Kilo

l liter

µg microgram

µl microliter

mg milligram

mililiter milliliter

mm millimeter

mmHg millimeters of mercury

M to molar

nm nanometer

Normal N

P weight

/ by

% percent

pH potential of hydrogen

x multiplication sign

v volume

When in the present norm it is mentioned to the Regulation, it must be understood that one is the Regulation of the General Law of Health in the matter of Sanitary Control of Activities, Establishments, Products and Services.

5. Classification

5.1 For effects of this norm the allowed natural organic colorantes are:

5.1.1 In foods

Oil of carrot (*Daucus carota*, L.);

Achiote, annato (extract of seeds of orellana *Bixa*);

Saffron (estigmas of *Crocus sativus*, L.);

Beta-Apo-8'-Carotenal;

Dehydrated Betabel;

Beta-carotene;

Cantaxantina;

Color caramel;

Chlorophyll;

Cochinilla (Extract of *Coccus cacti*, carmine L. or);

Cúrcuma (dust and oleoresina of rizoma of *Curcuma longa*, L.);

Extract of grape color (grape concord) (*Antocianina*);

Extract of tegumento of grape (Enocianina);

Flour of seed of cotton, cooked, toasted and partially desgrasada;

Fruit juices;

Vegetable juice;

Pimentón in dust (*Capsicum annum*, L.) Paprika;

Oleoresina of pimentón;

Riboflavina;

Riboflavina-5-phosphate;

Apocarotenoico Ester, and

Xantófilas.

5.1.2 In products of perfumería and beauty

Achiote, annato (Extract of seeds of orellana *Bixa*);

Beta-carotene;

Cochinilla (Extract of *Coccus cacti*, L., or carmine);

Color caramel;

Guaiazuleno;

Guanina, and

Henna

6. Sanitary specifications

The natural organic colorantes must fulfill the following physical, chemical specifications, of identity and purity:

6.1 Physical and chemical

6.1.1 Oil of carrot

Synonymous: *Daucus carota*, L.

6.1.1.1 Purity:

Hexano; not more than 25 mg/kg

Total of fatty acids; not less than 85%

Value of Iodo; from 118 to 134

Value of saponificación; from 165 to 185

Insaponificable matter; not more than 14%

Isopropílico alcohol; not more than 100 mg/kg

6.1.2 Achiote, Annato

6.1.2.1 Physical

Synonymous: CI Natural Orange 4; Lebensmittel. Nr. 3 Rocou; Bixina; Norbixina; Orleans; Orellana Terre

Class: Carotenoid

Number of Code: CI (1956) 75120

CI (1954) 1241

Schultz (1931) 1387

Qui'mico Name: The main colorante matter of the extracts of bija or achiote in oil is the carotenoid bixina. It is symmetrical the dibásico ester. Main the colorante matter of the watery extracts of bija is the alkaline salt of the norbixina.

Molecular Weight: Bixina: 394,52

Norbixina: 380,48

Color: Yellow (Orange in solution)

Solubility: The bixina is insoluble in ethanol water, slightly soluble and easily soluble in ether and oils.

Point of Fusion: Bixina: 198 °C

6.1.2.2 Purity

Arsenic (like Ace); not more than 3 mg/kg

Lead (like Pb); not more than 10 mg/kg

6.1.2.3 Identification

6.1.2.3.1 Espectrofotometría of absorption, in chloroform:

Bixina: Maximum to 503, 474.5 and 439 nm

E1%çm 2870 to 502 nm

Norbixina: Maximum to 509, 471.5 and 442 nm

E1%çm 2870 to 482 nm (in NaOH 0.1 N)

The bixina and the crocetina are both only carotenoid that do not oxidize easily by the oxygen of the air.

6.1.2.3.2 Chromatography in column:

Bija: The bixina is adsorbed energetically in the surface of alumina having formed a shining zone red-orange (unlike the crocetina). With metanol or ethanol they produce a change of color; of orange to yellow-orange.

With the reaction of Carr-Price, the zone of the bixina immediately becomes green-bluish (difference of the crocetina).

Bixina: Treatment of bixina crystallized with chloroform and reaction of Carr-Price. Green-bluish color is obtained.

Alkaline solutions of norbixina

The norbixina one forms a zone red-orange in the surface of the column.

6.1.3 Saffron

Synonymous: CI Natural yellow 6; Safran crocine; crocetina and croccus sativus.

6.1.3.1 Physical

Number of code: CI 75100

Colorantes main: crocina and crocetina

Molecular weight:

Crocina: 1 006,97

Crocetina: 328,41

Solubility:

Crocina: soluble in water, slightly soluble in absolute alcohol, insoluble glycerin and propilenglicol and in vegetal oils.

Crocetina: very little soluble in water and reliable organic, soluble in similar piridina and organic bases.

Point of fusion:

Crocina: 186 °C (with decomposition)

Crocetina: 285 °C (with decomposition)

6.1.3.2 Purity:

Ashes; not more than 8 %

Strange colors; negative

Maxima absorption between 464 and 434 nm

6.1.4 b-Apo-8'-carotenal

6.1.4.1 Physical:

Synonymous: CI Orange 6

Number of Code: CI 40820

Physical state: solid

Molecular weight: 416,65

Appearance: granular fine dust of color brown coffee

Point of fusion: 136-140 °C (with decomposition)

Solubility: Insoluble in vegetal oil and ethanol water, slightly soluble (until approximately 1.5%)

6.1.4.2 Purity:

Loss of weight by drying; not more than 0,2%

Remainders to the ignition; not more than 0,2%

Lead (like Pb); not more than 10 mg/kg

Arsenic (like Ace); not more than 1 mg/kg

6.1.4.3 Identity:

Espectrofotometría; not less than 96 nor more than 101% of C₃₀H₄₀O

Maxima absorption in ciclohexano; 456 to 485 nm

6.1.5 Betanina

6.1.5.1 Physical:

Synonymous: Rouge of betteraves

Class: Betanina.

Qui'mico Name: Betanina 1-[2-(2,6 dicarboxi-1,2,3,4-tetra-hidroxi-4-piridilideno) - etilideno]-5- b-D-glucopiranosil-oxi-6-hidroxindolium-2-carboxilato.

Molecular weight: 550,48

Description: Liquid, paste or dust red color or red brown.

Color: Red

Solubility: Easily soluble in absolute alcohol water, insoluble.

6.1.5.2 Purity:

Nitrate; not more than 2 anion g nitrato/g of red color

Arsenic (like Ace); not more than 1 mg/kg

Lead (like Pb); not more than 10 mg/kg

Mercury (like Hg); not more than 1 mg/kg

Heavy metals; not more than 40 mg/kg

Volatile material; not more than 4%

Insoluble acid ashes; not more than 0,5%

Content of expressed red color like betanina; not less than 0,4%

6.1.5.3 Identification:

Espectrofotometría;

pH 5,4: characteristic maximum to 530 nm

pH 8,9: vague maximum to 535 nm

E1%_{1cm} of the pure betanina to 535 nm in water: 475-625

6.1.6 B-Caroteno

6.1.6.1 Physical

Synonymous: CI Natural Yellow 26; CI Natural Brown 5;

CI Food Orange 5; Natural b-Carotene; Mixed Carotenes

Class: Carotene

Numbers of Codes: CI (1956) 75130

CI (1975) 75130

b-carotene CI (1975) 40800

Schultz 1403

Molecular weight: 536,89

Color: Reddish yellow to orange

Description: Red crystals in pure state

Solubility: The carotenes are insoluble in ethanol water, soluble and slightly soluble up to 1% in vegetal oils.

Point of fusion: to-carotene: 187°C

b-carotene: 176-182°C

G-carotene: 178°C

6.1.6.2 Purity

Arsenic (like Ace); not more than 3 mg/kg

Lead (like Pb); not more than 10 mg/kg

Loss of weight by drying; not more than 0,2%

Remainders to the ignition; not more than 0,2%

Espectrofotométrico test: from 96 to 101%

6.1.6.3 Identification

Espectrofotometría

E1%_{1cm} of b-carotene (trans) in hexano, maximum to 455-457nm and 482-486 nm.

Treatment with permanganato of potassium to 1% in alkali; they oxidize obtaining colorless substances.

An acetone b-carotene solution, after a treatment with a solution to 5% of sódico nitrito and sulfuric acid 1 N; there is carotene destruction and disappearance of color.

Chromatography: A zone for pure b-carotene is only obtained.

6.1.7 Cantaxantina

6.1.7.1 Physical

Synonymous: CI Food orange 8; b-carotene-4,4'-diona; Cantaxantina, and 4,4'-dioxo-b-carotene.

Number of code: CI (1975) 40850

Molecular weight: 564,86

Physical state: solid

Description: Crystalline dust of dark violet color

Solubility: He is soluble in insoluble acetone chloroform, very little soluble and in vegetal water, ethanol and oils.

Point of fusion: 207-212°C

6.1.7.2 Purity

Loss by drying; not more than 0,2%

Trans-cantaxantina and other carotenoid totals; not more of 5%

Lead; not more than 10 mg/kg

Arsenic; not more than 3 mg/kg

Mercury; not more than 1 mg/kg

Sulphated ashes; not more than 0,1%

Heavy metals; not more than 40 mg/kg

Remainders to the ignition; not more than 0,2%

Content; of 96-101% (expressed like cantaxantina)

6.1.7.3 Identification

Espectrofotométrico test:

A solution of cantaxantina in ciclohexano has a absorbancia between 468 and 472 nm.

6.1.8 Color caramel

6.1.8.1 Physical

Synonymous: CI Natural Brown 10

Physical state; dark or black coffee appears in liquid form of color and in solid form with characteristic scent to burned sugar and bitter flavor.

Solubility; soluble in ethanol water, slightly soluble, insoluble in ether, acetone and chloroform.

6.1.8.2 Purity

Lead (like Pb); not more than 5 mg/kg

Arsenic (like Ace); not more than 3 mg/kg

Mercury (like Hg); not more than 0.1 mg/kg

Ammoniacal nitrogen; not more than 0,5%

4-metil-imidazol; not more than 0,02%

Sulfur dioxide; not more than 0,1%

6.1.9 Chlorophyll

6.1.9.1 Physical

Synonymous: CI Natural Green 3; Lebensmittel-Grün Nr.1

Class: Forbina (equal to dihidroporfina).

Numbers of Codes: CI (1956) 75810

CI (1921) 1249a

Schultz (1931) 1403

Qui'mico Name: Chlorophyll (a): Magnésico complex of 1.3.5, 8 tetrametil-4- etil-2-vinil-9-ceto-10-carbo-metoxiforbifitil-7-propionato

Chlorophyll (b): Ceto-10-carbometoxiforbifitil-7-propionato magnésico complex of 1.5.8, trimetil-3-formil-4-etil-2-vinil-9-

Molecular weight: Chlorophyll (a) 893,54

Chlorophyll (b) 907,52

Color: green

Solubility: The chlorophyll is soluble in ethanol, ether, chloroform and benzene, insoluble in water.

6.1.9.2 Purity

Arsenic (like Ace); not more than 3 mg/kg

Lead (like Pb); not more than 10 mg/kg

6.1.9.3 Identification

Reaction in brown phase (hidróxido treatment with of potassium to 10% in metanol); a brown color takes place, returning quickly to its original color.

Reaction with ethanol; a green-blue color with intense red fluorescence takes place.

6.1.10 Carmine Cochinilla or

6.1.10.1 Physical

Synonymous: CI Natural network 4: Lebensmittel- Rot. Nr. 2.

Cochenille; Acide carminique, Carmine.

Class: Antraquinona.

Numbers of Codes: CI (1956) 75470 CI (1924) 1239 Schultz (1931) 1381

Qui'mico Name: Alkaline salts and alumínicas lacquers of carmínico acid (the colorante principle)

Molecular Weight: Carmínico Acido: 492,40

Color: Red

Solubility: Their alkaline salts are soluble in water and dissolvent etanólicos.

6.1.10.2 Purity

For the case of cochinilla

Arsenic (like Ace); not more than 1 mg/kg

Lead (like Pb); not more than 10 mg/kg

pH; not less than 5.0 and not more than 5.5 to 25 °C

Protein (N X 6.25); not more than 2,2%

Total solids; not less than 5.7 and not more than 6,3%

Methylic alcohol; not more than 150 mg/kg

Carmínico Acido; not less than 1,8%

For the case of the Carmine one

Arsenic (like Ace); not more than 1 mg/kg

Lead (like Pb); not more than 10 mg/kg

Volatile matter (to 135 °C by 3 hours); not more than 20%

Ashes; not more than 12%

Carmínico Acido; not less of 50%

6.1.11 Cúrcuma

6.1.11.1 Physical

Cúrcuma (Dust and oleoresina of rizoma of *Curcuma longa*, L.)

Synonymous: CI Natural Yellow 3; Lebensmittel - Gelb 6

Cúrcuma; Curcumine

Safran DES Indes; Indian saffron

Class: Di-cinamoil methane

Numbers of Codes: CI (1956) 75300

CI (1924) 1238

Schultz (1931) 1374

Qui'mico Name: Curcumina (main colorante); 1,7-bis-(4-Hidroxi-3-metoxifenil)-1,6-

heptadieno-3,5-dieno

Other colorantes:

Curcumina Dimetoxi: 1-(4-hidroxifenil)-7-(4-hidroxi-3-metoxifenil)-hepta-1,6-dieno-3,5-diona

Curcumina Bis-dimetoxi: 1,7-bis (4-hidroxifenil) hepta-1,6-dieno-3,5-diona

Molecular weight: Curcumina: 368,39

Curcumina Dimetoxi: 338,39

Curcumina Bis-dimetoxi: 308,39

Color: Yellow

Solubility: The curcumina is soluble in ethanol and glacial, insoluble acetic acid in water and ether.

Point of Fusion: Curcumina: 183 °C

6.1.11.2 Purity

Arsenic (like Ace); not more than 3 mg/kg

Lead (like Pb); not more than 10 mg/kg

Heavy metals; not more than 40 mg/kg

Ashes; not more than 10%

Humidity; not more than 6%

Content of curcuminoides expressed like curcumina; from 4% to 45%

Remainders of reliable:

Chlorinated hydrocarbons; not more than 3 mg/kg

Acetone; not more than 30 mg/kg

Isopropanol; not more than 3 mg/kg

Metanol; not more than 5 mg/kg

Hexano; not more than 2.5 mg/kg

6.1.11.3 Identification

Reaction of etanólico extract of cúrcuma with concentrated sulfuric acid; an intense crimson color takes place.

Test of boric acid; a coloration takes place red-cherry.

6.1.12 Extract of grape color (grape concord) (antocianina)

6.1.12.1 Physical

Synonymous: Anthocyanes; Anthocyanes (antocianinas)

Class: Salts of benzopirilo

Number of Code: Schultz (1931) 1394

Qui'mico Name: The antocianinas are glicósidos of salts of 2-fenilbenzopirilo, in their majority derived from hidroxilados. Aglucones of the antocianinas antocianidinas are denominated.

Molecular weight:

Pelargonidina: 306,72

Cianidina: 322,72

Peonidina: 336,74

Delfinidina: 338,72

Petunidina: 352,74

Malvidina: 366,77

Color: Red, violet or blue

Solubility: All the antocianidinas are soluble in water.

pH: 5,0

6.1.12.2 Purity

Arsenic (like Ace); not more than 1 mg/kg

Lead (like Pb); not more than 10 mg/kg

6.1.13 Extract of tegumento of grape (enocianina)

6.1.13.1 Purity

Lead (like Pb); not more than 10 mg/kg

Arsenic (like Ace); not more than 1 mg/kg

6.1.14 Flour of cooked, toasted seed of cotton and partially desgrasada.

6.1.14.1 Purity

Arsenic (like Ace); not more than 0.2 mg/kg

Lead (like Pb); not more than 10 mg/kg

Content of gopipol frees; not more than 450 mg/kg

6.1.15 Oleorresina of paprika

6.1.15.1 Physical

Synonymous: Extract of paprika

Class: Carotenoid

Solubility: Very soluble in vegetal oils; little soluble in glycerin water, insoluble.

Chemical name:

Capsaicina: N [(4-hidroxi-3-metoxifenil)metil] -8-metil-6-nonanamida; trans-8-metil- N-vainillil-6-nona-nanamida; N-(4-hidroxi-3-metoxibencil)-8-metil-non-trans-6-enamida.

Colorantes main:

Capsantina: 3,3'-b-dihidroxi, kapa-carotene-6'-ona, (3s, 3's, 5R, 5'r)

Capsorubina: Kappa, kappa-carotene-6,6'-diona,3,3'dihidroxi,(3S, 3S', 5R, 5'R)

Molecular weight:

Capsaicina: 305,40.

Capsantina: 584,85.

Capsorubina: 600,85.

Description: Viscous liquid of dark violáceo red color

6.1.15.2 Purity

Capsaicina; not more than 0,5%

Arsenic; not more than 3 mg/kg

Lead; not more than 10 mg/kg

Heavy metals; not more than 40 mg/kg

Remainders of reliable:

Acetone; not more than 30 mg/kg

Isopropanol; not more than 30 mg/kg

Metanol; not more than 50 mg/kg

Ethanol; not more than 50 mg/kg

Hexano; not more than 25 mg/kg

6.1.15.3 Identity:

Espectrofotometría: Maxima absorption to 470 nm in hexano.

6.1.16 Riboflavina

6.1.16.1 Physical

Synonymous: Lactoflavín; Lactoflavina; B2 vitamin

Class: Iso - aloxacina

Qui'mico Name: 7,8-dimetil-10-(1,-D- ribitol)-iso-aloxacina

Molecular weight: 376,37

Color: Yellow

Solubility: Barely soluble in water, insoluble in ethanol, ether, chloroform and acetone

Point of fusion: 280 °C approximately

6.1.16.2 Purity

Arsenic (like Ace); not more than 3 mg/kg

Lead (like Pb); not more than 10 mg/kg

Loss by drying; not more than 1,5%

Remainders to the ignition; not more than 0,3%

Content of C₁₇H₂₀N₆O₆ calculated in dry base; not less than 98% and not more than 102%.

Colorante, lumiflavina subsidiary material. It passes the test.

6.1.16.3 Identification

Specific rotation: [α]_{20D} of -120 to -135°

Espectrofotometría: Maximum of Riboflavina 220 to 225, 266, 374 and 444 nm.

Reaction with sódico ditionito: disappearance of an intense green-yellowish fluorescence.

Specific rotation: [α]_{25D} = -112 to -122° (50 mg in 2 milliliter of alcoholic solution of NaOH 0.01 N diluted with 10 H₂O to milliliter).

Determination of Lumiflavina in the presence of chloroform free of ethanol.

6.1.17 Riboflavina-5-phosphate

6.1.17.1 Physical

Class: Aloxazina

Description: Crystalline dust of color yellow-orange

Chemical name: Monosódica salt of 5-monofosfato ester of riboflavina

Solubility: Soluble in ethanol water, insoluble

Molecular weight: 547,37

6.1.17.2 Purity

Free phosphate; not more than 1%, calculated like PO₄

Free Riboflavina; not more than 6%

Loss to the drying; not more than 7 %

pH in solution 1:100; between 5-6,5

Remainders to the ignition; not more than 25%

Difosfato of riboflavina; not more than 6%, calculated like riboflavina

Content not less of the equivalent one of 75% of riboflavina

Lumiflavina, passes the test (C₁₇H₂₀N₆O₆)

Aromatic primary amines; not more than 70 mg/kg calculated like aniline

6.1.17.3 Identification

Specific rotation [α]₂₅^D between +37.0 and +42.0 calculated in dry base.

6.1.18 Apocarotenoico Ester

6.1.18.1 Physical

Solubility: Little soluble in oils and fats

Appearance: Viscous oil of coffee-reddish color

6.1.18.2 Purity

Value of peroxide; maximum 10%

Lead; not more than 10 mg/kg

Apocarotenoico ester content; minimum 20%

6.1.18.3 Identification

Absorption Maxima 447-449 nm

6.1.19 Xantófilas

6.1.19.1 Physical

Synonymous: Cantaxantina; flavoxantina; criptoxantina; luteína, filoxantinas; rubixantinas; rodoxantina; violaxantina; zeaxantina.

Class: Carotenoid

Numbers of Codes: CI (1924) 1249a

Schultz (1931) 1403

Qui'mico Name: Cetónicos or hidroxílicos derivatives of carotenes.

Molecular Weight: Luteína; 568,85

Physical state: solid

Color: Yellow

Solubility: Xantófilas is soluble in greasy and almost insoluble dissolvent fats and in petroleum ether.

Point of fusion: Flavoxantina: 184 °C Luteína: 190 °C

Rubixantina: 160 °C Violaxantina: 207 °C

Rodoxantina: 219 °C Zeaxantina: 215,5 °C

6.1.19.2 Purity

Arsenic (like Ace); not more than 3 mg/kg

Lead (like Pb); not more than 10 mg/kg

Mercury (like Hg); not more than 1 mg/kg

Loss by drying; not more than 0,2%

Remainders to the ignition; not more than 0,2%

Total of carotenoid, other different ones from trans-cantaxan-

bathtub, not more than 5%

6.1.19.3 Identification

Espectrofotometría not less than 96 nor more than 101% (luteína)

6.1.20 Guaiazuleno

6.1.20.1 Physical

Chemical name: 1,4-dimetil-7-isopropil-azuleno

Point of fusion: 30,5-31,5°C

6.1.20.2 Purity

Lead (like Pb); not more than 20 mg/kg

Arsenic (like Ace); not more of 3mg/kg

Mercury (like Hg); not more than 1 mg/kg

Total color; not less than 99%

6.1.21 Guanina

6.1.21.1 Physical

Synonymous: CI Natural White 1

Number of Code: CI 75170

Description: crystalline material

Molecular weight: Guanina 151,13

Hipoxantina 136,11

6.1.21.2 Purity

Remainders to the ignition; not more than 2 %

Lead; not more than 20 mg/kg

Arsenic; not more than 3 mg/kg

Mercury (like Hg); not more than 1 mg/kg

Total content of purinas; not less than 96%

Guanina; not less than 75%

Hipoxantina; not more than 25%

6.1.22 Henna

6.1.22.1 Physical

Synonymous: CI Natural Orange No. 6

Number of Code: CI 75480

Color: Orange to red

6.1.22.2 Purity

Humidity; not more than 10%

Total ashes; not more than 15%

Insoluble acid ashes; not more than 5%

Lead (like Pb); not more than 20 mg/kg

Arsenic (like Ace); not more than 3 mg/kg

6.2 Microbiological

Colorantes the object of this norm must be exentos of pathogenic microorganisms.

7. Sampling

The procedure of sampling for products object of this norm must subject to which it establishes the General Law of Health.

8. Methods of test

For the verification of the chemical specifications that settle down in this norm, the indicated methods of test in the Section of references are due to apply.

For the specific determinations the indicated Methods of test in normative Appendix A are due to apply.

9. Labeled

The label of products object of this norm, besides to fulfill established in the Regulation and the corresponding Mexican Official Norm, must subject to the following thing:

Index color (in its case);

Chemical name;

Declaration of purity;

10. Package and packing

10.1 Package

The products object of this norm are due to package in containers of sanitary type, elaborated with innocuous and resistant materials to different stages of the process, in such a way that they

do not react with the product or they alter the physical characteristics, chemical and organolépticas.

10,2 Packing

Envelopes of resistant material are due to use and that offers the suitable protection to the packages to prevent their outer deterioration, simultaneously that facilitates their manipulation, storage and distribution.

11. Control

Each lot of production must be endorsed by a certificate of analysis of the producer and leaf of identity with the specifications established in this norm. This information will be to disposition of the consumer who asks for it.

12. Agreement with international norms

This norm does not have agreement with international norms.

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14. Observance of the norm

The monitoring in the fulfillment of the present norm corresponds to the Secretariat of Health.

15. Use

The present Mexican Official Norm will take effect with its character of obligatory the thirty following days from its publication in the Official Newspaper of the Federation.

Effective Suffrage. NonRe-election.

Mexico, D.F., to 10 of May of 1995. - The Chief of a main directorate, Jose Meljem Moctezuma. - Heading.

Normative Appendix A

To METODOS OF TEST

All the reagents used in these tests must be analytical degree, unless another thing is indicated. When it is spoken of water must be understood distilled water.

1. Oil of carrot

1,1 Determination of the iodine index

Method of the iodine-chloride

1.1.1 Procedure

To place the sample in matraz of iodine, dry and to add 20 mililiter of tetrachloruro of carbon and to dissolve. To add 25 mililiter of iodine-chloride solution, to cover matraz with the cork previously dampened with potassium reactive iodide solution, to let rest in a dark place to temperature of $25 \pm 5^\circ\text{C}$ during 30 minutes with occasional agitation. To add in order mentioned 20 mililiter of potassium reactive iodide solution on the cone of matraz, carefully to clear the cork and to along with rinse it the walls of matraz with 100 mililiter of water recently boiled, to almost shake and to tittle with solution 0.1 Ms of tiosulfato of sodium, using at the end of the degree indicating starch solution. To write down the mililiter consumed (a). Simultaneously to make a target of consumed similar way and to write down the mililiter like (b). The difference between the volumes in mililiter of solution 0.1 Ms of tiosulfato of sodium consumed by the target and the sample, multiplied by 1.269 and divided between the weight of the sample taken in g is the value of iodine. The weight approximated in g that must be used for the analysis can calculate dividing the superior limit of the value of iodine waited for between 20. If it is consumed more than half of the halogen available to repeat the test using smaller amount of sample.

1,2 Determination of the saponificación index

1.2.1 Procedure

To place in matraz with cork grinding of 250 milliliter from 1.5 to 2 exactly heavy g of the sample, to add 25 milliliter of hidróxido solution 0.5 N of of ethanol potassium. To assemble to matraz an suitable condenser, to warm up in a steam bath, to maintain to ebb tide during 30 minutes shaking by rotation the content of matraz. To add 1 milliliter of indicating solution of fenolftaleína and to value the excess of hidróxido of potassium with 0.5 solution hydrochlorate acid N (HCl).

In the same way to run simultaneously a test in target of reagents using the same amounts and valuing.

To calculate the index of saponificación by means of the following formula:

$$S = 28.05 \times (B - V)/m$$

where:

S = Indice of saponificación of the sample.

B = Milliliters of solution 0.5 hydrochlorate acid N spent in the valuation of the target.

V = Milliliters of solution 0.5 hydrochlorate acid N spent in the valuation of the sample.

28,05 = miliequivalentes of hidróxido solution 0.5 N of of potassium.

m = Peso in grams of the sample.

1,3 Determination of the peroxide index

1.3.1 Procedure

To weigh with exactitude an approximated amount to 5.0 g of the sample, to transfer them to matraz yodométrico of 250 milliliter, to add 30 milliliter of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, to shake until 0.5 dissolution and to add milliliter of potassium saturated iodide solution. To cover matraz and to let rest the mixture by 1 minute exactly, shake once in a while; to add 30 milliliter of water and holder gradually with solution 0.01 Ms of tiosulfato of sodium with vigorous agitation and to continue until almost the yellow color disappears, to add 0.5 milliliter of indicating starch solution and to continue the degree shaking vigorously until the blue color disappears.

To calculate the target of reagents.

To calculate the peroxide index by means of the following formula:

$$\text{Indice of peroxide} = 1000 \text{ Ms} \times (a-b)/m$$

where:

to = milliliter of solution of tiosulfato of sodium spent in the degree of the sample.

b = milliliter of solution of tiosulfato of sodium spent in the degree of the target.

M = molaridad of the solution of tiosulfato of sodium.

m = weight in g of the sample.

2. Achiote, annato

2,1 Determination of Soluble Annato in Oil:

2.1.1 Procedure

2.1.1.1 Method To Dissolve 0.1 g of the chloroform sample and to dilute with chloroform in matraz volumetric to 100 milliliter To determine the phantom of absorption of the sample, using a cell of 1 cm between 600 nm and 400 nm. To measure the absorbancia in the maximum of absorption near 503 nm and to 404 nm and to run a target with reliable.

$$[A_{503} + A_{404} - 0.256 (A_{503})] \times 100$$

% of pure color (like bixina) =

$$(c) 286.6 \times s \times 1 \times a$$

where:

To = Absorbancia of the solution problem to the indicated wavelength.

l = Length of the cell pattern (in cm).

to = Concentration of the sample (in g/l).

0,256 = Factor related to the absorbancia of bixina in nm and 503 chloroform to 404 nm.

282,6 = Absortibilidad of bixina to 503 nm (in l/g-cm).

2.1.1.2 Method B. Determinar the absorbancia running a target of a 467 to the maximum chloroform solution and shows nm. To calculate the percent of color like bixina being used 320 l/g-cm like the absortibilidad.

2,2 Determination of Soluble Annato in water:

2.2.1 Procedure

To dissolve 2 g of sample in watery solution of hidróxido of potassium to 5% and to measure the maximum of 480 absorbancia to nm.

To calculate the percent of purity of color like bixina being used 287 l/g-cm like the absortibilidad.

2,3 Determination of Annato, Emulsions:

2.3.1 Procedure

To dissolve to the chloroform sample 1:1 v/v. To fit acidifying with drops of glacial acetic acid, and to again measure the same absorbancia of reliable to the Maxima 500 absorption nm.

To calculate the percent of color purity, being used 287 l/g-cm like the absorptibilidad.

3. Saffron

3,1 Ash determination

In a capsule previously defective, 5 are weighed exactly saffron g and it is introduced in a stove of drying to temperature of 100-105°C, maintaining it until constant weight. The remainder is put under calcination to temperature nonsuperior to the red shade (around 525°C). The excess of weight on the tare of the crucible or capsule, multiplied by 20 gives the percentage of ashes.

3,2 Determination of strange colorantes

To deal with a small amount saffron with benzine. It does not have to yield color some; in case of doing it to the presence picric acid or derivatives of tar can be suspected. To boil a small amount of saffron with potassium cyanide and potash, the appearance of red color purple reveals the picric acid presence.

4. b-apo-8'-carotenal

4,1 Identification

To determine the absorbancia of solution 460 B prepared directly to 488 and nm. Radius A_{488}/A_{460} is between 0.77 and 0,85.

To determine the absorbancia of solution B to 460 nm and of the solution To 332 a nm. The radius $A_{332}/(10 \times A_{460})$ is between 0.063 and 0,075.

Solution To Transferir 40 mg of the sample, exactly heavy, within matraz volumetric of 100 mililiter, to dissolve in 10 mililiter of free acid chloroform, to take to the volume with ciclohexano and to mix. To take 2 mililiter from this solution with pipette and to place it in matraz volumetric of 50 mililiter, to take to the volume with ciclohexano and to mix.

Solution B. Tomar with a pipette 5 mililiter from the solution To and placing it in matraz volumetric of 50 mililiter, taking to the volume with ciclohexano.

4,2 Procedure

To determine the absorbancia of solution B in a cell of 1 cm in a maximum of absorption of wavelength of 460 nm with espectrofotómetro, using ciclohexano like target.

To calculate the amount in mg of C₃₀H₄₀O in the sample by means of the following formula:

$$\text{mg of } 25000 \text{ C}_{30}\text{H}_{40}\text{O} = A/264$$

in which To it is the absorbancia of the solution and 264 are the absorptibilidad of pure b-apo-8'-carotenal.

5. Betanina

5,1 Determination of percent of color in Betanina

5.1.1 Reagents:

Solution Buffer (pH 5)

5.1.2 Procedure

To dissolve to a heavy amount correctly in a solution buffer and to dilute to an advisable volume with the solution buffer (total V in milliliter); the Maxima absorption can be in the rank from 0.2 to 0.8. To centrifuge the solution if it is necessary, and to measure the absorption correcting with a target composed of solution buffer (pH 5).

The color content is calculated in the bases of the Maxima absorption A (to 530 nm) having used the specific absorbancia for betanina.

$$A_{1\%} = 1120$$

cm

To x V

% red color (c) =-----

1120 xs L x W

where:

To = absorbancia Maxima.

V = volume of measured proven solution in milliliter.

L = length of the cell measured in cm.

W = weight of sample in g.

5,2 Nitrate determination in Betanina

5.2.1 Reagents

Standard nitrate solution - (10.000 mg/l)

To dissolve 16.31 potassium nitrate g (KNO₃), previously dried to 105°C by 24 hours in 1 000 milliliter of water.

Solution buffer. To dissolve 6.66 octahidratado aluminum sulphate g, Al₂(SO₄)₃ 8H₂O, 3.12 silver sulphate g (Ag₂SO₄), 1.24 boric acid g (H₃BO₃) in 900 milliliter of water, to fit to pH 3.0 with sulfuric acid (H₂SO₄) 1 Ms and to dilute with 000 water to 1 milliliter.

Diluted solution buffer. To dilute to the standard solution with the diluída solution buffer, to prepare in order the following solutions: 0, 100, 200, 300, 400, 500 mg nitrato/l.

5.2.2 Procedure

To exactly weigh 0.5 g of the sample in matraz Erlenmeyer, to add 50 mililiter of diluted solution buffer and to dissolve with agitation.

To also measure the potential of the calibrated solution and of the solution problem.

To draw up to the calibration chart using antilogarithmic paper, marking in abcisas the nitrate concentration. Of the calibration chart, to read the nitrate concentration of the sample.

Calculations:

a

Nitrate content = ----- colorante g/g demateria

200 xs W x C

where:

to = nitrate Concentration of the sample, mg/l.

W = Peso of the sample.

C = % calculated red color.

6, b-carotene

6,1 B-carotene determination (Soluble in cold water)

6.1.1 Procedure

To weigh 120 exactly mg of the mixture in matraz volumetric of 250 mililiter To add 5 mililiter of distilled water and to deal with ultrasound to maximum about 60 °C by approximately 10 minutes. To cool the solution under a cold water obstacle, to add 100 mililiter of absolute ethanol, to mix and to take to volume with dietil ether. To centrifuge 50 mililiter of this cloudy solution by 5 minutes. To take an aliquot one from 5 mililiter of the solution clear and to place in matraz volumetric of 100 mililiter, to evaporate to dryness in a rotavapor to 50 °C.

After cooling, to wash the remainder with 1 mililiter of 100 absolute ethanol and to dilute to mililiter with ciclohexano (solution to measure). With espectrofotómetro with band of amplitude of approximately 1 nm, to measure the absorbancia of ciclohexano the final solution to 454 nm con, using quartz cells of 1 cm.

A454 x 5000

Content deb - carotene % =

2230 xs W

where:

A454 = Absorbancia of the final solution to 454 nm.

W = Peso of the sample.

2230 = Absorbancia of reference (1%, 1 cm).

5000 = Factor of dilution.

6,2 B-carotene determination in emulsion

6.2.1 Procedure

To weigh 100 exactly mg of the sample in matraz volumetric of 100 milliliter To add 25 milliliter of distilled water and to deal with ultrasound by 5 minutes to 20 °C. To add 2 milliliter of HCl 1 N, 5 milliliter of ethanol and 50 milliliter of chloroform. To shake by 20 minutes and to centrifuge by 10 minutes. To praise/pour off the sobrenadante solution, to take 5 50 milliliter from this one and to transfer it to matraz volumetric of milliliter and to take to the volume with ciclohexano. With espectrofotómetro adapted to measure the absorption of the final solution to 454 nm, using ciclohexano like target.

To calculate the content on the basis of and $1\% \zeta m = 2230$, which is the theoretical value corresponding to the radius of present isomerism in the product.

7. Cantaxantina

7,1 Sulphated ash determination

7.1.1 Procedure

To transfer 2 g of sample to a platinum crucible previously defective or another container of 100 appropriate material of 50 or milliliter and to add sufficient diluido H₂SO₄ to dampen the sample totally. To warm up smoothly using a grill or a burner, or an infra-red lamp, until the sample is perfectly dry, to continue warming up until all the sample has been carbonized and to cool. To dampen the remainder with 0.5 milliliter of concentrated acid H₂SO₄ and to warm up in the same way until the rest of the sample and the excess of H₂SO₄ have been volatilized.

Finally to incinerate in a muffle warming up to 800 ± 25 °C by 15 minutes or more if it is necessary, until completing the ignition, cooling in a desecador and grief.

(Note: In the period in which the volatilización of H₂SO₄ is promoted, it is advised to exactly add some ammonium carbonate fragments before completing the ignition).

8. Color caramel

8,1 Ammoniacal Nitrogen determination

8.1.1 Materials and reagents

2 mantillas of heating

2 matraces ball of 300 milliliter with meeting 24/40

2 Kjeldahl filters

Per them of boiling

2 230 hasty glasses of of mililiter

Hidróxido of sodium 0.5 N

Hydrochlorate Acido 0.05 N

I oxidize of magnesium

Red methyl indicator

Antiespumante

8.1.2 Procedure

To weigh 3.0 carefully sample g and to transfer it to matraz ball with 200 mililiter of distilled water. To add 2 magnesium oxide g, 2-3 drops of antiespumante and to per them of boiling.

To prepare the target of reagents, to place 200 distilled mililiter of water, 2 magnesium oxide g, 2-3 drops of antiespumante and to per them of boiling. In 2 hasty glasses of, to place in each one 25 mililiter of HCl 0.05 N with 2 drops of red methyl indicator, being these the receiving glasses. To connect matraz ball with the coolant and to make sure that the unloading of the coolant is below the acid level in the receiving glass. Simultaneously to work the sample and the target of reagents. To put under heating until by distillation approximately 100 have been collected mililiter of liquid.

To title the content of the receiving glass to pH 6.2 with NaOH 0.05 N. Calcular the percentage of ammoniacal nitrogen.

$$(\text{mililiter HCl} \times \text{HCl}) - (\text{mililiter Na OH} \times \text{N NaOH}) \times 1,4007$$

% of ammoniacal Nitrogen =

Weight of the sample

8,2 Determination of 4-metilimidazol

8.2.1 Materials and reagents

Gas chromatograph, equipped with hydrogen detector with ionization of flame, silanizada glass column empacada with 7.5% of carbowax 20 KOH Ms + 2% on a mesh 90/100.

Glass tube for chromatography 22 xs 300 mm with removable key of teflón

Matraces volumetric of 5, 10 and 50 mililiter

Plastic funnel 100 mm of diameter

Matraz ball of 300 milliliter

Glass of precipitated of plastic

Distilling equipment

Pasteur Pipettes

Fiber glass Pyrex

Rotatory evaporator to the emptiness

Diclorometano

2-metilimidazol

4-metilimidazol

Tetrahidrofurano or acetone

Hidróxido of sodium 3.0 N (to dissolve 120 g in 1 distilled water l)

Equivalent Celite 545 or

8.2.2 Procedure

8.2.2.1 Purification of reagents

Technique of external standard. To purify crystals of 4-metilimidazol reactive degree submissive distillation to the emptiness (boiling point 92-93°C and 0.05 mm Hg).

8.2.2.2 Standard preparation of solutions

To weigh 50 carefully mg of the reagent before purified to place in matraz privileged of 50 milliliter and to dissolve with tetrahidrofurano or acetone until obtaining the volume.

To take from each matraz aliquot of 0.25, 0.50, 1.0, 1.5, 2.0, 3.0, 3.5, 4.0 and 5.0 milliliter and to place them in matraces privileged of 10 milliliter each one, to add tetrahidrofurano or acetone until obtaining the volume. The prepared standard solutions represent concentrations of 25, 50, 100, 150, 200, 250, 300, 350, 400 and 500 mg/kg of 4-metilimidazol. To cool the solutions.

8.2.2.3 Sample preparation

To weigh 10.0 g of color and 5.0 hidróxido g of of sodium (NaOH) 3 N, to place them in the plastic glass, to mix perfectly. PH of the mixture does not have to be greater to 12.

To add 20.0 545 g of celite or equivalent and to mix with a stainless steel spatula until she is homogenous are to say that the color is uniform. The mixture is semidry. To plug with fiber glass Pyrex the inner part of the tube for chromatography of 22 xs 300 mm, to transfer totally the previous mixture with aid of the plastic funnel and to plug with fiber glass the superior part of the tube. To accommodate the content, leaving to fall the tube vertically until 10 cm of distance between the top have left and the empacada surface. The bed of the tube must have 15 cm of

height and uniform being to allow that the elución happens quickly. To rinse the glass with diclorometano (eluyente) and to flood the tube opening the key of teflón until the liquid the level of the key has left, to close the key and to leave the eluyente is in contact with the enemy with the bed during 5 minutes. To open the key and to collect in matraz 200 ball mililiter of the eluyente to a 5 flow of mililiter per minute. To remove the eluyente (diclorometano) submissive distillation to the emptiness to 35°C and 45-50 KPa, until reducing the volume to 1 mililiter.

Precaution: during the concentration passage, to observe matraz to make sure that it does not happen rupture of the same one.

With a Pasteur pipette, to transfer the extract to matraz measured of 5 mililiter, to rinse with 0.75 portions of mililiter of tetrahidrofurano until obtaining the volume. To mix perfectly, to this we will denominate sample to him.

8.2.2.4 Gas chromatography

Conditions of operation:

Temperature of the column 190 °C (isothermal)

Temperature injection port 200 °C

Temperature of the detector 250 °C

Acarreador gas Nitrogen

Flow 50 ml/minuto

Size of sample 5 µl

External standard technique

8.2.2.5 Determination of the standard curve

To inject 5.0 µl of each standard solution prepared with 4-metilimidazol and to obtain the chromatograms.

For each standard chromatogram, to calculate the corrected area of tip, multiplying the height of the tip (in mm) by wide to half of the height of the tip (in mm) by the corresponding attenuation and the factor depending on the apparatus in individual and used parameters of operation. Graficar the areas of tip corrected against its respective concentrations of 4-metilimidazol and to obtain therefore the curve. To inject 5.0 in the same way µl of the sample that the solutions standard. To calculate the area of the tip to interpolate to obtain the content of 4-metilimidazol in the sample.

8,3 Sulfur dioxide determination by the method modified Mornier-Williams.

8.3.1 Material and reagents

Equipment Mornier-Williams modified according to annexed figure 1

Hydrogen peroxide to 3% prepared recently

Red methyl indicator

Pirogálico Acido

Hidróxido of potassium

Hydrochlorate Acido 4 N

Hidróxido of sodium 0.05 N

Gas nitrogen

Antiespumante agent

8.3.2 Procedure

8.3.2.1 Sample preparation

To install the equipment as one is in figure 1.

Note: he is recommendable to have 2 mounted equipment, since one will be used for the sample and the other for the target of reagents.

8.3.2.2 Each receiving tube to add 10 milliliter of hydrogen peroxide to 3% and 3 drops of the red methyl indicator. To bleed the system with nitrogen during 5 minutes, to verify that there are not flights in the meetings and to use the clamps to assure the installation. In matraz of distillation to place 40 g of 400 sample and to use milliliter of distilled water to transfer it. To add a drop of antiespumante.

In a second matraz of distillation to prepare target being added 400 milliliter of distilled water and 1 drop of antiespumante.

To prepare the washing gas bottles adding to each one of them a solution of piroganol (4.5 g of piroganol dissolved in 15 milliliter of distilled water). To bleed the system with nitrogen during 15 minutes, to add a solution of KOH (65 dissolved KOH g in 85 milliliter of water) to each washing gas bottle and to bleed again with nitrogen. To add to matraces of distillation 90 milliliter of HCl 4 N while it is continued bleeding the system.

The nitrogen flow must be fit so that in the washing gas bottles a smooth and constant current of bubbles is observed. To provide water to the coolant and to put under heating matraces of distillation to cause ebb tide during 25 minutes (90 volts). When a continuous ebb tide is obtained to provide more heat (120 volts) and to reflujar during 105 minutes. To clear the water to coolants and to continue warming up until the first adapter shows something of condensation. To suspend to the heat provision and the nitrogen flow, to clear the receiving tube and to drain its content in a 250 glass of precipitated of milliliter To rinse the receiving tube with distilled water and to drain the water in the same glass of precipitated. To rinse each one of the parts of the system (cooling, adapter, first condenser and concentrator) with distilled water and to drain it in the glass of precipitated (to use approximately 175 milliliter). To title all the content of the glass of hasty with NaOH 0.05 N adding drops of red of methyl until pH 6,2 turns to clear yellow.

To calculate the percentage of SO₂ with the following formula.

mililiter of NaOH x N of NaOH x 3,205

% SO₂ =

sample g

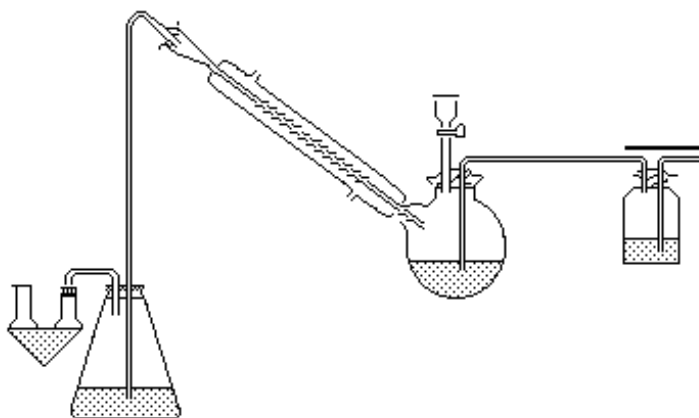


Figura 1

9. Carmine Cochinilla or

9.1 Carmínico acid determination

9.1.1 Materials and reagent

Measured Matraz of 1 l

Glass of precipitated of 250 milliliter

Hydrochlorate Acido 2 N

9.1.2 Sample preparation

Hidroliza the carmine red sample of with HCl freeing to all the carmínico acid and its absorbancia to a 494 wavelength of nm.

9.1.3 Procedure

To weigh 0.10 exactly g of sample in a glass of precipitated of 250 milliliter, to add 30 milliliter of HCl 2 N and to warm up to boiling in bath Maria during 3-4 minutes, quantitatively to drain this mixture in matraz measured of 1 l and to take to the volume with distilled water.

Prepare a target of reagents with HCl 2 N and water distilled partly proportional to the used thing to analyze the sample. To read the absorbancia of the solution in espectrofotómetro to a wavelength of 494 nm.

To calculate the percentage of carmínico acid according to the following formula:

$$T_o \times 100$$

% Acido carmínico =

$$13,9 \times s \times b \times W$$

where:

T_o = Absorbancia (read value to 494 nm).

b = Thickness of the cell.

13,9 = Factor.

W = Peso of the sample in g.

10. Cúrcuma

10,1 Determination of curcumina

10.1.1 Reagents

Acetone

Standard of curcumina for synthesis (C₂₁H₂₀O₆)

10.1.2 Preparation of the standard

To place 250 exactly mg of standard curcumina heavy in matraz volumetric of 100 mililiter, to write down the weight in mg like acetone w . Disolver, to take to the volume with acetone and to mix.

To take 1 mililiter from this solution and to place in 100 a volumetric second matraz of mililiter to take to the volume and to mix, finally to take with a 5 pipette mililiter of the previous solution, and to place them in matraz volumetric of 50 mililiter, to take to the volume with acetone and to mix.

10.1.3 Sample preparation

To place 250 mg of exactly heavy sample within matraz volumetric of 100 mililiter, to write down the weight in mg like acetone W . Disolver, to take to the volume with acetone and to mix. To take 1 mililiter from this solution and to place in a 100 second matraz of mililiter, to take to the volume with acetone and to mix finally, to pipetear 5 mililiter of the previous solution, and to place it in matraz volumetric of 50 mililiter, to take to the volume with acetone and to mix.

10.1.4 Procedure

To determine the absorbancia of each solution to 418 nm in a cell of 1 cm, using like white acetone.

To calculate the percent of curcumina in the sample with the following formula:

$$100 \times \frac{w}{W} \times \frac{A_u}{A_s} = \% \text{ of curcumina}$$

where:

A_u = Absorbancia of the sample.

A_s = Absorbancia of the curcumina standard.

w = Peso of the standard in mg.

W = Peso of the sample in mg.

Note: The absorbancia readings must be made more soon possible after being prepared the solutions to avoid losses in the color.

11. Flour of cooked, toasted seed of cotton and partially desgrasada

11,1 Determination of the content of gossypol frees.

11.1.1 Procedure

To weigh 1 g of the sample and to transfer it to matraz 250 Erlenmeyer of mililiter Covers the bottom of matraz with per them of glass of 6 mm of diameter. To transfer 50 mililiter of a acetone-water solution (7:3) to matraz, to cover and to shake in a mechanical agitator during 1 hour. Filter this solution through a paper dry filter of average porosity, receiving in matraz small and discard the first mililiter of the filtrate. To take 2 10 portions of mililiter from the filtrate and to deposit each one of these in matraces volumetric separately of 25 mililiter To take to the first portion to the volume with a isopropílico alcohol solution - water (8:2).

To the second aliquot one to add 2 mililiter of redestilada aniline and to warm up in bath Maria by 30 minutes. To warm up in bath Maria a solution containing 2 mililiter of aniline and 10 mililiter of a solution acetone - water (7:3), to clear both solutions of the bath, to cool to room temperature, and to take to the volume with a isopropílico alcohol solution - water (8:2).

To measure the absorbancia of the 2 solutions shows and the reagent against the isopropílico alcohol 460 a maximum near nm.

Calculate the corrected absorbancia (difference between the absorbancia of the samples, corrected with the target) and compare against prepared standards of similar way.

12. Oleoresina of paprika

12,1 Determination of capsaicina

12.1.1 Procedure

To weigh 5 exactly g in matraz, to add 100 milliliter of metanol to 70%, to shake by 30 minutes. To let rest the solution by 5 minutes and leak. To cover the funnel to avoid the evaporation. The first 25 milliliter of the filtrate discard and the rest of the filtrate is mixed well. To distribute this solution in matraces of 100 milliliter and to prepare them of the following way:

Matraz 1 Matraz 2 Matraz 3 Matraz 4

Filtered solution 4.00 milliliter 4.00 milliliter -- --

Distilled water 17.80 milliliter 16.80 milliliter 19.00 milliliter 18.00 milliliter

HCl 1 N 1.00 milliliter -- 1.00 milliliter --

NaOH 1 N -- 2.00 milliliter -- 2.00 milliliter

Certain Value A1 A2 A3 A4

The solutions are mixed in matraces with 100 milliliter of metanol. The absorbancia evaluates A1-A4, the 4 296 solutions are measured to 248 and nm (deuterium lamp, quartz cell).

Calculations:

$$[(A2 - A1) - (A4 - A2)] \times 2500$$

a) to 248 nm = % of capsaicina

$$314 \times s \times W$$

$$[(A2 - A1) - (A4 - A3)] \times 2500$$

a) to 296 nm = % of capsaicina

$$127 \times s \times W$$

where:

2500 = dilution.

314 and 127 = correction factors.

W = Peso of the sample in g.

Determinations retorts of (a) and (b) cannot differ more than 10%. This determination can be repeated.

12,2 Determination of SPEAR (American Spice Trade Association). To evaluate the color according to the following procedure:

12.2.1 Materials and reagents

Espectrofotómetro, able to measure the absorbancia to 460 exactly nm

Square cells of absorption of 1 cm with cork

Matraces volumetric with cork of vidro grinding

Glass pipettes of 10 milliliter

Paper glassine

Paper filter equivalent Whatman no. 40 or

Acetone reactive degree

Sulphate crystals of ammonium and cobalt

Potassium dichromate reactive degree

The sulphate of ammonium and cobalt must dry by one week in a desecador containing anhydrous calcium sulphate. A preliminary treatment for potassium dichromate is not necessary.

Standard solution of color: 0,3005 g/l of potassium dichromate, more 34.96 g/l of ammonium sulphate crystals and cobalt in a H₂SO₄ solution 1.8 Ms. The absorbancia of this solution in a cell of 1 460 cm to nm could be 0,600.

12.2.3 Procedure

To exactly weigh a sample from 50 to 80 mg in matraz volumetric of 100 milliliter and to take to the volume with acetone. To do the extraction in a minimum time of 15 minutes, shaking occasionally. With a pipette of 10 milliliter, to transfer 10 milliliter of the extract in volumetric other matraz of 100 milliliter and to take to the volume with acetone. To filter the diluted extract using paper Whatman no. 40; to reject 10 to 15 milliliter of the first filtrate. To praise/pour off a portion of the filtrate in a cell and to measure the 460 absorbancia to nm, using acetone like target.

To determine the absorbancia of the standard solution of color to 460 nm.

Calculations:

$$C = \frac{A}{l} \times 164 \times W$$

C =

W

where:

C = Value of extracted color SPEAR.

To = Absorbancia of acetone extract to 460 nm.

W = weight of the sample in g.

l = 0,600/As length of the cell and factor of correction of the instrument.

Ace = Absorbancia of the standard solution of color.

12.3 Determination of reliable residual

This procedure is for the determination of remainders of acetone, dicloruro of ethylene, hexano, isopropanol, metanol, diclorometano and tricloroetileno.

12.3.1 Materials and reagents

Head of distillation

Designed Clevenger trap to be used with oils heavier than the water. Design in figure no. 2

Tolueno. The tolueno used for this analysis does not have to contain some of the solvents to determine by this method. The purity can be determined by gas chromatography using one of the following columns or its equivalent ones:

17% in weight of UCON 75-H-90 000 on a mesh 35/80

20% of UCON LB-135 on a mesh 35/80

15% of UCON LB-1715 on a mesh 60/80 or a mesh of 50/60

Follow the conditions described in the procedure and inject the same amount of tolueno like the inyectora in the analysis of the solvents. If the impurities interfere with the test they will appear as present tips before the tip of the tolueno and could be removed by divided distillation.

Benzene. The benzene used for this analysis must be free of impurities that interfere; the purity can be determined as it were described for tolueno.

Detergente and Antiespumante

Free volatile compound products must be used. If these compounds are present, can be removed by prolonged boiling of the watery solutions of products.

12.3.2 Solution of reference A

G/kg of benzene prepares a solution of tolueno containing 2.5. If the tolueno available contains benzene as only impurity the level of this last one can be determined by gas chromatography and adds sufficient benzene until reaching the level or the 2.5 concentration of g/kg.

12.3.3 Solution of reference B

Prepare a solution containing 0.63% of water acetone.

12.3.4 Preparation of sample A (all the solvents except metanol)

G of the sample, 1 mililiter of the solution of reference places 50 To, 10 sodium sulphate g (Na_2SO_4) anhydrous, 50 mililiter of water and one small amount of detergent and antiespumante in matraz of round bottom with a rough glass neck (of grain) of 20/40. Fix to the head of the neck a filter with a condenser cooled with water of 400 mm and a container in which mililiter of the distilled one recovers approximately 15. Potassium carbonate g adds to distilled the 15 (K_2CO_3)

anhydrous, shakes while it cools off and allows the separation of phases. All the solvents except metanol will be present in the phase of tolueno, which is used in the procedure. Separate the watery phase to use it in the sample preparation B.

12.3.5 Sample preparation B (only metanol)

Mililiter with the rough glass neck places the obtained watery phase of the sample preparation To in matraz of distillation of round bottom of 50 24/40, adds a few stones of boiling and 1 mililiter of the solution of reference B and to recover approximately 1 mililiter of the distilled one, which will along with contain something of metanol of the sample acetone like an internal pattern (of reference). The distilled one is used in the procedure.

12.3.6 Procedure

Use an equipment of gas chromatography with an electrical detector and a system of injection of sample or injection in column. In typical conditions, the instrument contains a column of 63 mm by 183 to 244 cm, maintained isothermally between 70 and 80 °C. The rate of flow of the acarreador gas is from 50 to 80 ml/minuto and the 20 sample size is from 15 to μl (for the electrical detector).

The columns To, B, Cs and D as they are described in tolueno can be used as it follows:

To This column it separates acetone and metanol of his respective watery solutions. It can be used to separate and to analyze hexano, acetone even ethylene in the phase of tolueno from the sample preparation A. The elución order is acetone, metanol and water or hexano, acetone, isopropanol, diclorometano, benzene, tricloroetileno and dicloruro of ethylene in tolueno.

B This column separates diclorometano and isopropanol. The elución order is hexano more acetone, diclorometano, isopropanol, benzene, tricloroetileno in tolueno.

C This is the best column for general intentions, except for the determination of metanol. The elución order is hexano, acetone, benzene in tolueno.

D This column is used for the determination of metanol which eluye just before the great water tip.

Calibration.

Determine the answer of the detector for radios known reliable by means of the injection of mixtures known reliable and benzene in tolueno. The levels of reliable and benzene in tolueno must be of the same magnitude that the presents in the sample under analysis.

Calculation:

Calculate the areas of the solvents with respect to the benzene and later it calculates the factor of calibration F as it follows:

Weight of the reliable Area of the benzene

F (reliable) = x

Weight of the benzene Area of the solvent

The recovery of several solvents of the sample with respect to the recovery of the benzene is as it follows:

Hexano 52%

Acetone 85%

Isopropanol 100%

Diclorometano 87,5%

Tricloroetileno 113%

Metanol 87%

Calculate mg/kg of reliable residual (except metanol) by the following formula:

$$43.4 \text{ reliable} \times F \times 100 \times \text{Area of the solvent}$$

Reliable residual = x

$$\% \text{ of the solvent recovered} \times \text{Area of the benzene}$$

where:

43,4 = mg/kg of the internal pattern of related benzene to 50 g of sample for analysis.

Calculate mg/kg of metanol residual by the formula:

$$100 \times F \times \text{Area of metanol}$$

Reidual Metanol = x

$$0.87 \times \text{Area of acetone}$$

where:

100 = Concentration in mg/kg of the internal pattern of acetone related to a sample of 50 g.

F = Factor of calibration for metanol determined by the use of a well-known mixture of metanol and acetone.

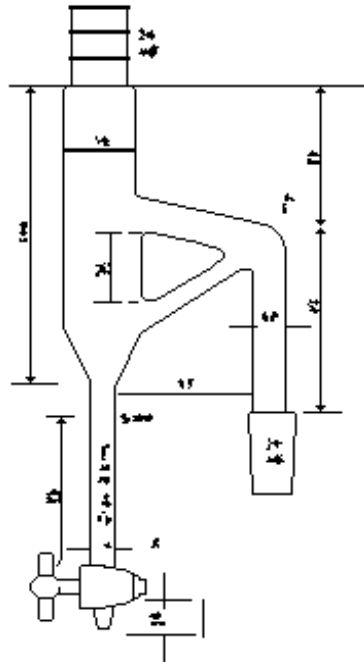


Figura 2

13. Riboflavina-5-Fosfato

13,1 Phosphate determination

13.1.1 Reagents

Preparation of the standard

To transfer 220 mg of monobasic 1000 potassium phosphate KH_2PO_4 to matraz volumetric of milliliter, to dissolve and to take to the volume with water and to mix. To transfer 20 milliliter of this one solution to matraz volumetric of 100 milliliter, to take to the volume with water and to mix.

Preparation of the test

To transfer 300 mg of the sample to matraz volumetric of 100 milliliter, to dissolve, to take to the volume with water and to mix.

Acid solution of molibdato.

To dilute 25 milliliter of a solution of molibdato of ammonium (7 g of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in sufficient water to do 100 milliliter) in 200 milliliter of 25 slowly water and adding milliliter of 7.5 H_2SO_4 N.

Ferrous sulphate solution

Just before being used, milliliter of 7.5 H_2SO_4 N prepares a watery solution to 10% of ferrous sulphate containing 2 percent milliliter of final solution.

13.1.2 Procedure

To transfer 10 separately milliliter of the standard preparation and the preparation of test in matraces 50 Erlenmeyer of milliliter, to add 10 milliliter of the acid solution of molibdato and 5 milliliter of the ferrous sulphate solution to each matraz and to mix.

To determine the absorbancia of each solution in a cell of 1 700 cm to nm with espectrofotómetro, using as white a mixture of 10 milliliter of water, 10 milliliter of the acid solution of molibdato and 5 milliliter of the ferrous sulphate solution. The absorbancia of the test solution is not greater than the one of the preparation of the standard.

13,3 Determination of free riboflavina and riboflavina difosfato

13.3.1 Preparation of the standard

To transfer 35 mg of standard riboflavina of reference in matraz Erlenmeyer of 250 milliliter, to add 20 milliliter of piridina, 75 milliliter of water and to dissolve the riboflavina with frequent agitation. To transfer the solution to matraz volumetric of 1 000 milliliter and to take to the volume with water and to mix. To transfer 20 milliliter of this solution to a volumetric second matraz, of 1 000 milliliter, to fit pH to 6.0 with the addition of 8 milliliter of 0.1 H₂SO₄ N, to take to the volume with water and to mix. Finally, to transfer 25 milliliter of the last solution to matraz volumetric of 100 milliliter, to take to volume with a dioxano mixture - water (1:3), and to mix. This solution contains 0.175 µg of riboflavina by milliliter.

13.3.2 Solution buffer

To dissolve 15.6 monopotassium phosphate g (NaH₂PO₄·2H₂O) in 100 milliliter of water, to add 59.3 hidróxido milliliter of of sodium 1 2000 N and to dilute to milliliter with water. To verify pH with a potentiometer and to fit to 7 if it is necessary.

13.3.3 Sample preparation

To dissolve to 100 g of the sample in 10 milliliter of solution buffer pH 7. Prepare a strip of Whatman paper chromatography type 3 mm, of coefficient of average landslide or another equivalent in paper electroforesis and to saturate the paper with solution buffer of pH 7. Using a micropipette to apply 0.01 milliliter of the solution shows throughout the narrow line of the side of the cathode, being the paper of the side that contains the sample. Apply a potential of approximately 250 volts, it leaves electroforesis it it continues by 6 hours and it removes the paper of the camera.

To detect some free or difosfato riboflavina of riboflavina, observing the line the light of the day or under ultraviolet light. If it exists riboflavina it frees, it will appear like a band near the line of beginning, and the difosfato of riboflavina will appear like the remotest line of the line of beginning.

Precaution. The riboflavina is destroyed if it is exposed to the ultraviolet light by more of seconds.

Court the respective bands, placing them in matraces Erlenmeyer of 250 milliliter separately, containing 35.0 milliliter of a dioxano-water mixture (1:3), and allowing that becomes stabilized until the spots of the strips are eluyan completely.

13.3.4 Procedure

Using fluorómetro available, it determines the intensity of the fluorescence of each solution shows and of the standard a 530 length of nm, using a wavelength of 460 excitation of nm.

The fluorescence of the solutions containing the riboflavina and the difosfato of riboflavina, will not be greater than the produced ones by the standard preparation.

13.4 Determination of lumiflavina

13.4.1 Reagent

In order to prepare the standard, to dilute in matraz volumetric of 1 000 milliliter, 3 milliliter of potassium dichromate 0.1 N and to take to the volume with water.

Pass something of chloroform through an alumina column to remove any rest of ethanol. For 10 milliliter of this chloroform, adds 35 mg of the sample, shakes 5 minutes and leaks. The color of the filtrate does not have to be more intense than the one of 10 milliliter of the standard preparation when it is seen in identical containers.

13.4.2 Procedure

Make the test under tenuous light. In matraz volumetric of glass color to ambar of 500 milliliter, dissolves 10 mg of sample in 100 milliliter of 2.5 water and adds milliliter of glacial acetic acid and in matraz volumetric of 500 milliliter to take to the volume with water. Milliliter of this solution in matraz volumetric of glass places 20.0 color amber, adds 3.5 milliliter of a solution 1.4% p/v of 200 sodium acetate and to take to milliliter with water.

Nm measures the absorbancia (a) to a maximum of 444.

To x 5000

% Total of colorantes matters = x 1,367

328 xs W

Where: To = Absorbancia of the sample to 444 nm.

W = Mass of the sample in g.

13.5 Aniline determination

13.5.1 Procedure

To dissolve 20 g of the sample in 400 milliliter of to 5 water and to add milliliter of NaOH 1 N. Agitar in a separation funnel and to separate with 4 50 successive portions of milliliter of chloroform, every time during 5 minutes. With 400 successive portions of milliliter of NaOH 0.1 N, to wash the reunited clorobencénicos extracts until the superior watery layer is colorless. To filter the clorobencénica solution. To take 75 milliliter from clorobencénico extract to shake with 2 50 successive portions of milliliter of HCl 0.5 N, and soon with 2 25 successive portions of milliliter of water. To neutralize the watery extracts reunited with a solution of NaOH to 30%; soon to acidify with 10 milliliter of HCl 0.5 N. Disolver in this solution of 1-2 g of potassium bromide. After cooling in frozen water to add around 20 drops of sodium nitrate 0.1 N and to let rest during 10 minutes. To eliminate the excess of nitrate adding acid aminosulfónico. To spill the solution in about 5

mililiter of a solution to 3% of salt sódica R(sal of naphthol-2-sulfonic-3,6 acid) added with 10 mililiter of NaOH 2 N. Dejar rest during 15 minutes. To acidify the solution of colorante in the presence of the red Congo (indicating), until this one turns to the blue one and to leak. The aminobencénico colorante will not happen.

To add water to the filtrate up to 200 mililiter; soon to measure the extinction to 490 nm (E4).

13.5.2 Calculation

$$E4 \times 266$$

Aniline content =

$$(\text{mg/kg}) 2.26 \times s d4$$

1 mg/ml

And 490 for aniline = 226

1 cm

14. Guanina

14,1 Determination of guanina and hypoxanthin

14.1.1 Materials and reagents

Matraces volumetric of 1 000, 500 and 100 mililiter

Fiber glass filters 934 AH

Matraz Erlenmeyer of 250 mililiter

Crucible Gooch No.4

Plate to warm up with magnetic agitator

Magnetic bar to shake

Porous average glass filter

Glass of precipitated of 200 mililiter of long form

Matraz of suction

Glass of clock

Filter Fisher

Glass agitator

50 pipettes of 5 and milliliter

Ultraviolet Espectrofotómetro covering a range with phantom of 300 nm to 230 nm with scale of wavelength able to read to 1 nm and absorbancia with an exactitude of 1% of absolute error

Cells of quartz absorption of 1 cm

Solution carbonate-bicarbonate buffer

To dissolve 13.44 sodium bicarbonate g (reactive degree) and 45.75 anhydrous distilled water sodium carbonate g and to dilute to a liter

Land filter of diatomaceas, previously washed with water-acetone (1:1) and dried

Acetone suprapure degree

Solution of hidróxido of sodium 1 N

Anhydrous crystals of guanina

Anhydrous hypoxanthin crystals

Preparation of the target

In matraz volumetric of 100 milliliter to dilute to 5 milliliter of NaOH of sodium 1 N with water, to take to the volume and to mix. To transfer an aliquot one of 10 milliliter to matraz volumetric of 1 000 milliliter, to add to 50 milliliter of solution carbonate-bicarbonate buffer and to take to the volume with water.

14.1.2 Preparation of the standard

To weigh exactly 150 separately mg of anhydrous crystallized guanina (dried to 105°C) and 136 mg of hypoxanthin (dried to 105°C) in two matraces volumetric of 100 milliliter. To add to 5 milliliter of NaOH 1 N and to warm up in steam bath to facilitate the dissolution. To cool and to take to the volume with water. To transfer an aliquot one of 10 milliliter to matraz volumetric of 1 000 milliliter to add to 50 milliliter of solution carbonate-bicarbonate buffer and to take to the volume with water.

To measure the absorbancia of each 259 final solution to 273 and nm in cells of 1 cm against the target.

To calculate and 1% ϵ_{cm} evaluating both wavelengths for both standards. To make several determinations and to remove to the value average to be used in the calculations. Once the coefficient of extinction of the standard has been determined, this one needs to be verified periodically.

14.1.3 Sample preparation

To exactly weigh an appropriate amount of sample (1g for a 10% of mixture) of the colorante (mixed perfectly) within a glass of precipitated of long form of 200 milliliter To add acetone to disperse the approximately 4 well sample and to filter g. Mezclar. To leak through a crucible Gooch no. 4 containing fiber glass filter. To wash the glass of precipitated and to leak perfectly with acetone while it is filtered, allowing to happen completely before washing again. To repeat 4

times the washing with acetone. Then to wash the glass of precipitated with water to remove some acetone remainder.

14.1.4 Procedure

Quantitatively to transfer the filtrate to the bottom of a glass of precipitated of long form of 200 milliliter, to place a bar of magnetic agitation. To wash to the crucible perfectly with water, adding the water of washing to the glass of precipitated. To add from 80 to 90 milliliter of water and to remove the material filtered with agitation. To add to 5 milliliter of NaOH 1 N, to cover with a clock glass, to warm up magnetically to boiling shaking by 90 minutes. With the aid of the water and a filter Fisher, to filter the hot sample through a porous glass filter being contained an aid filter, within matraz 250 Erlenmeyer of milliliter To wash the glass of precipitated and the glass filter perfectly with water until the volume of the filtrate is sure approximately from 100 to 150 milliliter.

Quantitatively to transfer the filtrate with the help of water to matraz volumetric of 500 milliliter, to cool and to take to the volume with water.

To measure the absorbancia of the solution to 273 nm in a cell of 1 cm against the target.

Calculations:

Coefficient of extinction of the standard

$$A$$

$$E_{1\% \text{ } 1\text{cm}} =$$

$$C \times l$$

where:

To = Absorbancia to specific wavelength.

C = Concentration % (p/v).

l = Wavelength in cm.

Concentration of guanina and hypoxanthin in the solution problem:

$$(A_{273} \times E_{h259}) - (A_{359} \times E_{h273})$$

$$C_g =$$

$$(E_{g273} \times E_{h259}) - (E_{g259} \times E_{h273})$$

$$(A_{259} \times E_{g273}) - (A_{273} \times E_{g259})$$

$$C_g =$$

$$(E_{g273} \times E_{h259}) - (E_{h259} \times E_{h273})$$

where:

Cg = Concentration of guanina in % (p/v).

Ch = hypoxanthin Concentration in % (p/v).

To = Absorbancia to specified wavelength.

Eg = Coefficient of extinction of the standard of guanina to specified wavelength.

Eh = Coefficient of extinction of the standard of hypoxanthin to specified wavelength.

Guanina and hypoxanthin in the sample:

$$C_g \times DF$$

% Guanina (p/p) =

$$w$$

$$C_h \times DF$$

% Hipoxantina (p/p) =

$$w$$

where:

Cg = Concentration of guanina in the solution problem.

Ch = hypoxanthin Concentration in the solution problem.

DF = Factor of dilution in milliliter.

w = Peso of the sample in g.