Search for food rich in Provitamin D:

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Index:

1. SUMMARY:

It has been made a Provitamin D2 extraction (Ergosterol) with vegetal samples by some physicochemical procedures made in a chemical analysis laboratory. The samples selected were lettuce, date, grape, rice and mushroom. Once the analysis is completed, compare how many Provitamin D2 has each one of them.

2-INTRODUCTION/DESCRIPTION OF THE INVESTIGATION:

In this project we have chosen some samples of vegetable foods like lettuce, rice, dates, grapes and mushroom. We have made a number of analysis with them in order to see the amount of Provitamin D2 in each sample. To achieve that, first we aisled the Provitamin in each sample using an extraction procedure with hexane. Then it was measured in a laboratory instrument called High Performance Liquid Chromatography (HPLC). With this system we were able to know the exact amount of Provitamin D2 in each kind of food by means of a comparison method (calibration) of the results of the samples with references made by us in the laboratory as of pure substances (patterns). Finally, after making all the measurements in the chromatography, we made a number of mathematical calculations (we prepared a calibration line of 5 points), which allow us to know the amount of provitamin in each food.

3. PURPOSE:

The purpose of this project is to know the amount of provitamin D2 that has each sample and compare them to release what food between the ones we have chosen is richer in this precursor of vitamin D.

As a result of that, we can recommend to those people who have a deficit of this vitamin, that a good and healthy way to prevent the ingestion of vitamin supplements is to eat some foods permanently and usually to eat an amount of these fresh foods.

4. PLANNING AND OBJECTIVES:

Our investigation consist of, by some process and manipulation of the samples realise what of the foodstuff we have chosen has more amount of provitamin D2.

That processes can be divided in three parts:

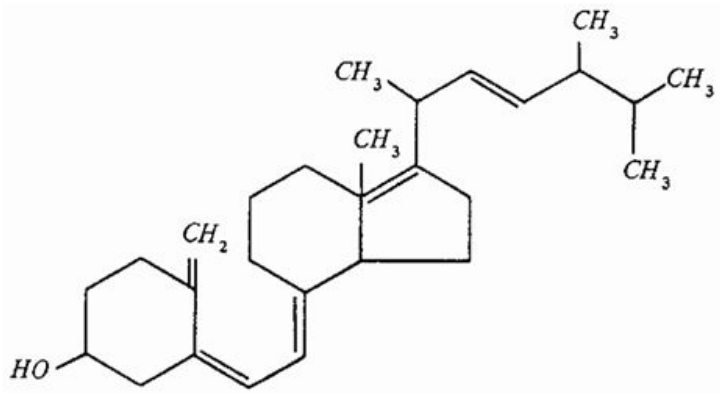
1- Previous preparation of the samples to aisle the Provitamin in each one and make that process with hexane.

2- Make a set of disolutions that have the same substances as the ones we will analyse (we will analyze the vitamin D2 (calciferol), the provitamin D2(ergosterol) and the vitamin D3(cholecalciferol)) made from the pure substances(patterns) that have been bought, in different ranks or concentrations.

3- Measure all the disolutions and samples on the chromatography system and afterwards purchase the raw results for a following mathematical treatment.

5. THEORETICAL BASICS:

The existence of the vitamin D in food was discovered in 1922 by Elmer McCollum. He observed that, when liver oil of cod was treated with oxygen, an essential substance was destroyed (this substance was then called the " factor A") but another substance stayed, which had an anti-rickets effect. Inasmuch as it was the fourth vitamin known, it was called vitamin D. The preventive effect of sunlight on rickets had been already described by Armand Trousseau in 1861. After the knowledge of the relation between rickets and vitamin D, scientist were able to establish that this substance was produced on the skin by the action of sunlight, and they could even radiate some foods with light UV. In the 30s, Adolf Windaus discovered the chemical structure of vitamin D and its relation with sterols. He was given the Nobel prize in 1928, the first one in which " vitamins" were mentioned.



Vitamina D
(calciferol)

The Vitamin D is a fat-soluble vitamin with a steroid chemical structure. It can be obtained from foods that have it and we can also synthesize it on the skin from cholesterol and the radiating action of ultraviolet light from the Sun. It is heat-resistant (until 150ºC) and it's fairly resistant to oxidation, although it can be oxidized when it is unsaturated.It is susceptible to sunlight action.

The vitamin D is necessary for the development, growing and maintenance of bones. It´s more important function is to maintain the calcium homeostasis. It takes action in the calcium absorption in the intestine. When the amount of calcium is not enough for the body's requirement, the vitamin D sends a signal to osteoblasts that makes them dissolve the calcium in bones, regulating homeostasis.
That is why we need to ingest a notable amount of foods with provitamin D, like between 800-1000 UI, but there are few foods that contain it. Also, in lots of cases synthesizing this provitamin with ultraviolet radiations of sunlight it is still not enough to get the recommended levels. For this reason, we will always need supplements or a bigger ingest of foods that contain this vitamin.

The deficit of this vitamin causes health problems that can be very serious, like rickets or osteomalacia (disorder in the mineralization of bones, caused by low levels of vitamin D or phosphate), also it can increase the risk of hypertension, diabetes, autoimmune diseases and cancer.

There are lots of studies that compare the vitamin D2 and the vitamin D3. The majority of them (but not all), find that the efficacy of vitamin D3 in the human body is equal or superior, but never lower. That is why, in fortified foods and nutritional supplements, we should look for cholecalciferol (vitamin D3). Also, supplements with vitamin D3 make our blood maintain the correct levels of vitamin D for a longer time,

which is a benefit during seasons with a low incidence of sunlight in very northern and southern regions.

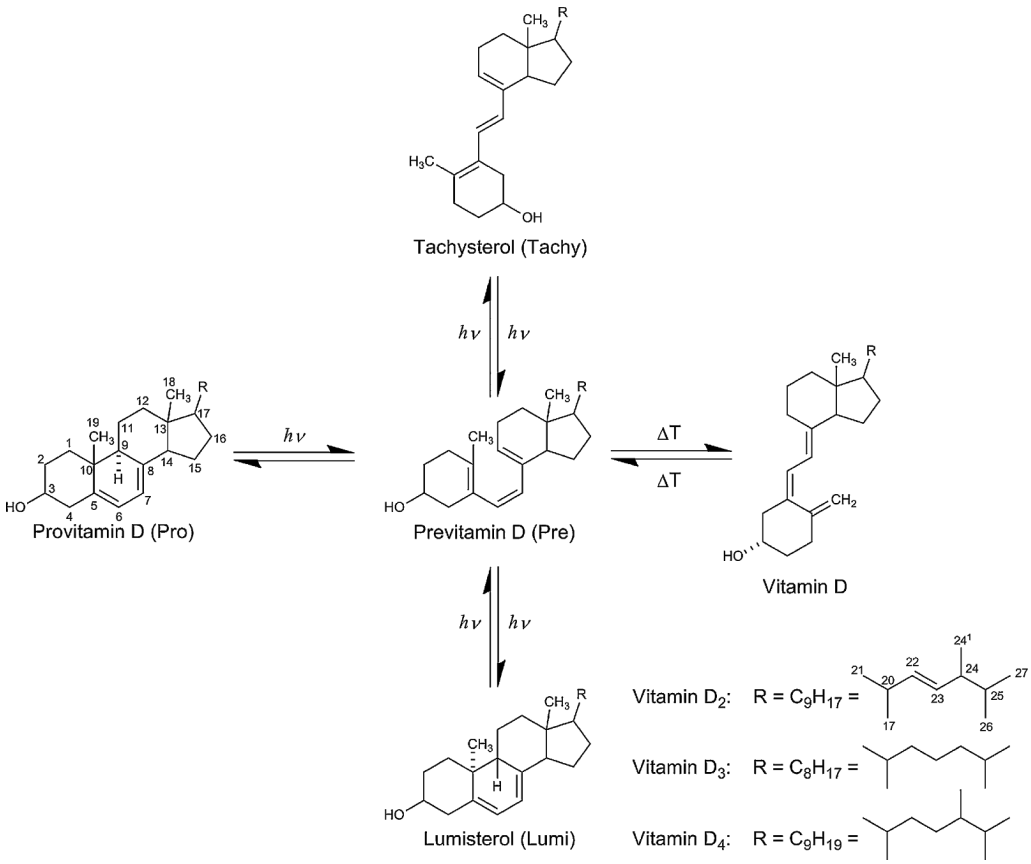
With regard to pharmacological treatment, both vitamins have shown a similar efficiency against diseases related to deficit of vitamin D, although there are studies which point that vitamin D3 is more efficient and has a bigger therapeutic range before toxicity appears. That is the reason why vitamin D3 is the one used as a medication.

If we use sunlight as the source of vitamin D, we have to expose our skin during a period of time that depends on its colour. Pale skins only need between 10 and 15 minutes in a small surface (like the face or forearms). If the surface is bigger, the period of time decreases. After 20 minutes, there is not conversion of provitamin D, so a longer exhibition is not necessary. In people with a darker skin, this period of time increases between three and six times. The organism of a person with a very dark skin needs like an hour to synthesize enough vitamin D.

During winter, if the ultraviolet radiation is not enough, our body cannot synthesize the vitamin, so it has to use its reserves. This is called “Winter of vitamin D”. Only adults can do it, children aren’t able to store this vitamin.

There are two types of provitamin D that our body can synthesize: provitamin D3 (7-dehydrocholesterol), which has an animal origin, and provitamin D2 (ergocholesterol), that has a vegetable origin. Ultraviolet radiation transforms this provitamins in cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) respectively.

However, plenty of foods with an animal origin and foods with a vegetable origin have provitamin D2, for example fish, eggs, mushrooms, etc. But when we eat this foods, provitamin D3 doesn’t transform directly in vitamin D2, it needs to be synthesized.
Putting as an example the mushroom, as we mentioned it in the biography, it has been proved that we can find provitamin D (Pro) in mushrooms, and with ultraviolet light this provitamin becomes previtamin D (Pre), which becomes vitamin D with the heat action. But it has a negative effect: during the procedure, toxic substances like taquistherol (Tachy) and lumisterol (Lumi) are generated from previtamin D (Pre). These toxic substances are harmful for people if they are ingested in big quantities.



6- EXPERIMENTAL DEVELOPMENT

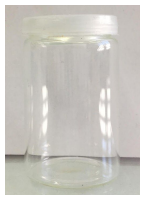
6.1- FOODS USED

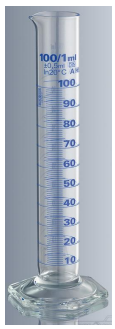
We have used samples of: lettuce, rice, mushrooms, grapes and dates

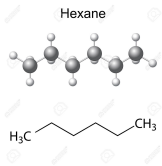
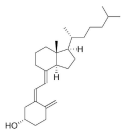
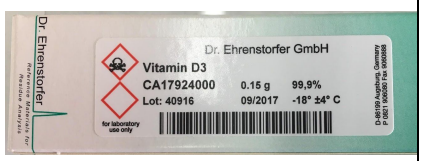
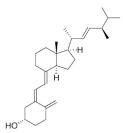
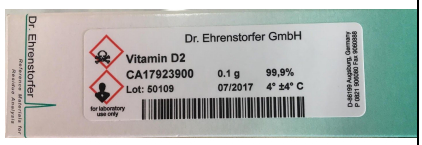
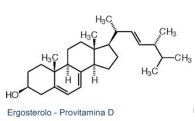
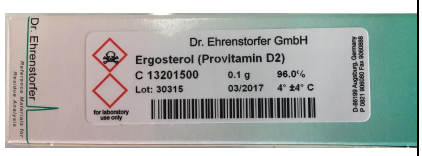




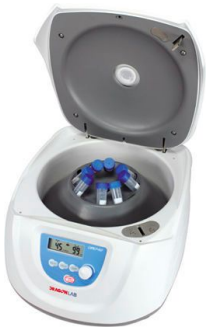
-MATERIALS AND REACTIVES

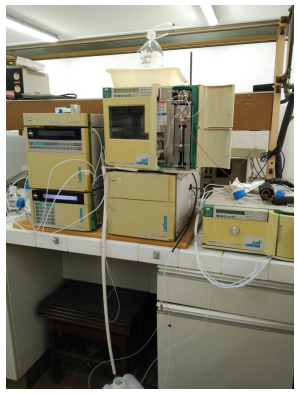
Spatulas	
Microvial of injection	
Precise analytical balance	
Breker of 50 mL	
Glass vial of 10 mL	
Foil	

Flask of 10mL	
Test tubes	
Coffee grinder	
Micropipette of 10-100 µL and 100-1000 µL	
Cleaning bottle	
Microfilter with syringe of 0.45µm	
Falcon tube of 15 mL	

Parafilm	
Formic acid	
Methanol	
Hexane  .	
Vitamin D3  Colecalciferol Vitamina D3	
Vitamin D2  Ergocalciferol Vitamina D2	
Provitamin D2 (Ergosterol) 	

6.2- INSTRUMENTATION MATERIALS:

Ultrafreezer	To freeze the samples and conserve them during a long period of time.	
Lyophilizer	It is used to remove the water of products or dissolutions using low pressure and temperature. It is composed of three parts: -Dry chamber or lyophilizer chamber: where the substance is placed. -Condenser with refrigeration circuit: it is communicated with the dry chamber. It is where the steam produced is condensed. -Vacuum system	
Centrifuge	It is a laboratory equipment that generates rotation movements. It separates the substances according to its density.	
Vacuum Manifold	It prepares or concentrates samples for its use in the HPLC, HPLC-MS or GC-MS. A vacuum pump and a nitrogen bullet can be joined to it. It can be used to evaporate solvents	

High performance liquid chromatography (HPLC) with a ultraviolet detector system	It can separate, quantify and identify the components of a mixture. It is composed by a high pressure pump, an automatic injector and a ultraviolet detector system.	
Ultrasound-bath	This system is used to homogenize samples. The inside of the bucket (that is full of water) vibrates at a certain frequency.	
chromatographic separation column (Synergi 2,5 u Hydro-RP 100 A 100×3.00 mm 2,5µ)		

6.3-EXPERIMENTAL METHODS:

We chose some foods that, according to the bibliography are rich in provitamin D. This foods were: lettuce, mushroom, grape, rice and date. We made two samples of each food, so we had 10 samples. First, we prepare the samples making some basic procedures like: cleaning,cutting and freezing them in the ultrafreezer (at -80ºC). Then the water has to be removed using the lyophilizer (it makes low pressure and temperature on the samples). Some times if the food has a lot of water it is necessary to lyophilize it two times.
Finally, the samples are crushed with a coffee grinder, having as a result a thin dust.

After doing this, the samples have to be weighted in an analytical balance: 0.5 g of each sample (although it is impossible to weight the exact number). Then, 6 ml of hexane and 1 ml of vitamin D3 (internal standard) are added to the samples. This mixture is shaken by hand during 5 minutes, then it is centrifuged at 3200 g during

15 minutes. The result is a liquid substance (supernatant) and a solid substance at the bottom of the Falcon tube. The supernatant is drawn off to the glass vial of 30 ml. It has to be covered by aluminium foil to avoid the degradation of provitamin D2 (which is susceptible to sunlight). Hereafter, 6 ml of hexane have to be added again to the solid substance, and the process explained before is repeated. The supernatant obtained in this second extraction is put in the same vial as in the first extraction.
Finally the organic solvent (hexane) has to be evaporated by a nitrogen stream. The samples are reconstituted with 1 ml of methanol, and they are exposed to ultrasounds for 15 minutes (the objective of putting them in ultrasounds is to homogenize the samples). The samples are microfilter with a microfilter with syringe of 0.45 um.

Then, the patterns of a calibration line that will be measure in the HPLC have to be made. To do this, each vitamin has to be in a pattern dissolution with a concentration of 100 ml/L. With this pattern dissolutions, a intermediate dissolution that contains ergosterol and vitamin D3 is prepared, and with this one diluted dissolutions with concentrations of: 0.5; 1; 2.5; 5 and 10 mg/L are made.

6.4- EXPERIMENTAL MEASUREMENT INSTRUMENTATION:

The optimal conditions for each parameter of the instrument are needed for the measurement in the chromatographic system. The separation of the different substances we want to analyze (ergosterol, vitamin D2 and vitamin D3, being this last one our internal standard) is performed by the “separation gradient”, which consist of putting a mixture of dissolutions and solvents through a separation column, changing the proportions with the time. In this way each substance arrives at the end of the system at a different time, so we can analyze them separately though in the first dissolution were all mixed. In our case, we applied the separation gradient that can be seen in the table:

Time (min)	% Water + 0,01% of Formic Acid	% Acetonitrile	Flow (mL/min)
0	1	99	0,5
4	1	99	0,5
5	10	90	0,5
8	10	90	0,5

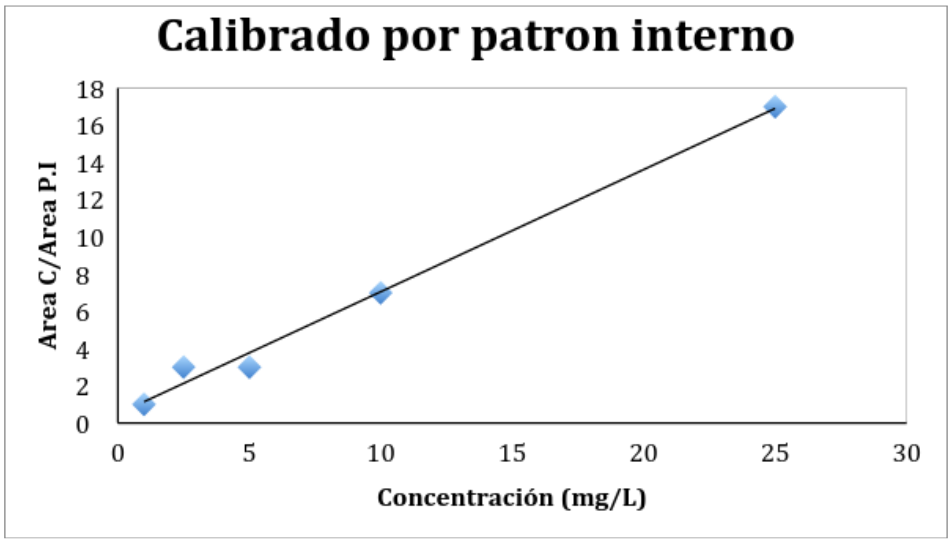
8,1	1	99	0,5
10	1	99	0,5

When the substances have completely crossed the column, they arrive at the place where its light absorption is measured. This place is called detector, in our case it was a visible-ultraviolet detector. Each substance absorbs light with a different wavelength, so we can identify the substance. In the next table there are the elution times (the time that takes each substance to cross the chromatography), which are characteristic for each substance.

Substance	Elution time (min)	Wavelength (nm)
Cholecalciferol (Vitamin D3)	5,23	250
Ergocalciferol (Vitamin D2)	5,36	255
Ergosterol (Provitamin D2)	7,31	270

6.5-RESULTS TREATMENT:

Calibrating is to establish references (with a known concentration and prepared by us: 0.5;1; 2.5; 5 and 10 mg/L) to know the areas that correspond to each concentration. There are different methods to calibrate: direct calibrate or internal standard calibration. We used the last one. For this method, a reference with a constant and known concentration is needed, in our case it was vitamin D3 of 1 mg/l. If the concentration is always the same, the area should be always the same, but it varies between injections. This variation allow us to correct the areas of the rest of injections and to detect unwanted variations in our procedure . It represents it in the calibration graph (Substance's area/ internal standard's area vs substance's concentration):



Thanks to microsoft excel we can see the equation of the line the data makes is . This is a statistical procedure called: “Least squares adjustment” . The equation obtained is : y = ax + b. This means, Area= pending·C + ordinate, where C is the concentration of the substance in mg/L. In our case, this equation is:

$$y = 0{,}6568x + 0{,}4857$$

Once the calibration is made, the concentration of ergosterol in each measured sample has to be calculated. With the areas of vitamin D3 and the area of ergosterol obtained in each sample, grape, lettuce, date, rice and mushroom, we can make this expression:

$$\frac{Area_{Ergosterol}}{Area_{Internal\ Standard}} = Pending \times Concentration_{Ergosterol} + ordinate$$

The last thing to do is to replace the data in the expression and to obtain the concentration of each substance in each sample in mg/L.

6.7-FINAL RESULTS:

According to the treatment of the data described in the last point, the results obtained are exposed in the next table:

Sample	Average content in ergosterol(mg/g) dry extract	% Moisture	Content in ergosterol (mg/g) in sample
Rice	<2	14	<0,28
Grape	<2	81,6	<1,62
Date	<2	73	<1,46
Lettuce	1890,5 ± 16	94	1191,0 ± 10
Mushroom	3790,9 ± 213	63	3563,5 ± 201

The content of ergosterol in sample was calculated knowing the percent of moisture of each food.

7-CONCLUSIONS:

Between all the samples used in this project, we verified that lettuce and mushroom are the foods with more concentration of ergosterol in their composition. As we see, the concentration of the mushroom sample is 3563,5 ± 201 mg of ergosterol in each gram of mushroom, while in lettuce this concentration is 1191,0 ± 10 mg of ergosterol in each gram of lettuce.

With this information we can conclude that the regular ingestion of lettuce and mushroom can help us to avoid the deficit of Vitamin D without using any kind of vitamin supplement, in a healthy and natural way.

8-SOCIAL TRANSFERENCE:

Thanks to conferences, these knowledge can be explained to people so they know about the Deficit of Vitamin D and the foods that contain the provitamin. There are a lot of people with this deficit that do not even know they have it. Informing them about it and about these foods can help them to increase its consumption and to prevent or solve this deficit in a natural a healthy way.

9- PERSONAL OPINION:

The experience of approaching to the investigation world and to develop this project by ourselves (always with the help and collaboration of the investigators and the teacher) has been very rewarding.

10- APPRECIATIONS:

To the investigators Rut and Julia and the teacher Rocío for helping and teaching us every time we needed it.
To the teachers of our high schools for informing us about this project and for sacrificing part of their time for us.
To the project Jóvenes con Investigadores for giving us this great opportunity to approach us to the investigation world.

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