PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

MOHAMED BOUDIAF UNIVERSITY - M'SILA

FACULTY OF SCIENCE

DEPARTMENT OF MICROBIOLOGY & BIOCHEMISTRY

N°:

FIELD: SCIENCES OF NATURE AND LIFE

SECTOR: BIOLOGICAL SCIENCE

OPTION: APPLIED BIOCHEMISTRY

Thesis presented for the award of

Academic Master's degree

By: DAMMANI LEILA

CHERRID MEFTAH

Title

Formulation of a dietary supplement, Madeleine enriched with vitamin D from cod liver oil.

Defended before a jury composed of:

Dr BELBAHI Amine	University of Msila	President
Dr BOUAOUDIA-MADI . N	University of Msila	Supervisor
Dr AOUN Omar	University of Msila	Examiner

Academic year: 2022/2023

THANKS

Above all, we thank Almighty God for giving us the will, strength and patience to complete this work.

First of all, I would first like to thank my thesis supervisor, Dr BOUAOUDIA- Madi Nadia, for her patience, availability and, above all, her judicious advice, all of which helped to fuel my thinking

We would like to thank all the professors and staff of the Biochemistry and Microbiology Department, headed by department head Dr. RAHALI Abdallah, for their efforts over the years.

I would also like to thank the entire teaching staff at Mouhamed Boudiaf msila University, and the professional lecturers responsible for my training: the head of alhadina, Professor zaouch Rida, and the heads of the mdm lahouaw Amel and ms Yasmine laboratories.

all the staff of the biology laboratory headed by sghiri Kamel and all the lab supervisors.

I would also like to thank all the members of the jury, Dr. AOUN and Dr. BELBAHI who honored me by agreeing to evaluate my dissertation.

I would like to thank all the people who contributed to the success of my internship and helped me write this thesis

Dedecation

If the first path is how many, the last will realize a dream. If the first start is a tear, the end is a stroke and each start of the end. And here are the years and the dream has come true. Thank you before you are satisfied and thank you if you are satisfied and thank you after satisfaction because you are ready to complete this work beyond. I dedicate this work

I have those who proudly bear its name to those who harvest the thorns in my path to open the way for me by the flag to my dear father Ismail". After God's merit for what I am today, my father comes back to me, a man who didn't receive even a fraction of what we have today.

And the man who strived all his life to be better than me the invisible hand of my precious mother Dalila is said to be behind every great man a woman and I say behind every successful woman I did not great walk and tired on all my steps and success and I endured every moment I lived and my support is weak in your God blessings for us. To Sindi and my fixed rib that doesn't lean on loneliness, my brother Younes.

To my love Flowers House, my sister Ghafran and Rym. To my older brother's shoulder who has stood by me through thick omar kettab

To my second mother, my young aunt, my dear, my first supporter, Aziza, and her chick daughter, Loujain.

To my uncle Samir and his wife Somaya.

To my memory colleague, my brother Meftah.

To Chahinez baatouch with Jawhar Fella's thirst....

To the two most wonderful people in the world, all the words in the world are not described by my twin soul Raouf and my brother who was not born of my mother Yassin....

To all those who had a hand in my memoir, to all those who shared with me the moments of writing and presence.

Leila

I couldn't have done it without God's help.

To the one who received the letter, showed honesty and advised the nation...

To the Prophet of Mercy and Light of the Two Worlds

His name is proud to reap the thorns of my path to pave the way for science after God's credit, what I am returns to the one who gave me and still gives me infinitely the perpetuation of God.

Invested in "My dear father".

To him who saw her heart before her eyes, and whom she embraced, cradling it before her hands to the shadow that sheltered it.

When God saved her, my beloved mother.

To my sisters and my big brother Salim and Allah in their life and performance for me and to all those who helped and supported me in this way to all those who shared with me in university life.

My teachers, friends and colleagues Al Tahir .rabie. Elias .Samir.

Meftah .

Summary

ملخص

يعتبر هذا الاختراع المكمل الغذائي الأول في العالم، الذي يحتوي على كمية كبيرة ومركزة من الفيتامين د المستخلصة من مواد طبيعية 100% دون أي إضافات أو مواد كيميائية .

هذا المكمل سهل وبسيط التصنيع بمكونات طبيعية ومتوفرة ذات قيمة غذائية كبيرة ومناسبة لجميع الفئات العمرية التي تعاني من هشاشة العظام، وبهذا المنتج يمكننا الاستغناء عن الأدوية المصنعة كيميائيا، والتي لها اثار وأعراض جانبية مثل الكبسولات.

لقد أجرينا العديد من الابحاث ولكن لم يتم إيجاد منتوج مشابه لمنتوجنا عدا كبسولات d3 الصناعية حيث أن اختراعنا يختلف اختلافا تاما عن كل الأدوية المصنعة حيث انه طبيعي 100% سهل التصنيع اقتصادي وفي متناول جميع فئات المجتمع .

بعد الدراسة المعمقة للسوق الجزائري والعالمي واجراء عدة بحوثات عبر الانترنت لم نرصد أي منتوج لمنتوجنا فقررنا ان نكون السباقين في هذا المجال من خلال صنع كعك غني بالفيتامين D3 والعديد من المركبات الطاقوية مثل الكاليسيوم والحديد والعديد من الفيتامينات الأخرى مثل : A ,c ، والبروتينات وهذا من أجل الوقاية جسم الانسان من الامراض وبناء جسم صحي ومتكامل .

Abstract

This invention is the first dietary supplement with a high concentrated amount of vitamin D3 extracted from 100% natural substances without additives or chemicals.

This supplement is easy and simple to manufacture with natural and available ingredients of high nutritional value suitable for all age groups suffering from osteoporosis and with this product we can do without chemically manufactured drugs that have the most side symptoms like capsules.

- Previous industrial technical status :

We have done a lot of research, but no product similar to our product has been found except for industrial D3 capsules where our invention is completely different from all manufactured drugs because it is 100% natural and easy to manufacture economically and accessible to all segments of society.

- Purpose of the invention:

After studying the Algerian and international market in depth and conducting several online searches, we did not follow any product of our product. We decided to be the races in this field by making cakes rich in vitamin D3 and many energy compounds such as calcium, iron and many other vitamins such as A, C, proteins and it is for the human body to prevent diseases and build a healthy and integrated body.

Résumé

Cette invention est le premier supplément alimentaire au monde avec une grande quantité concentrée de vitamine D extraite à 100% de substances naturelles sans additifs ni produits chimiques

Ce supplément est facile et simple à fabriquer avec des ingrédients naturels et disponibles d'une grande valeur nutritionnelle adaptée à tous les groupes d'âge souffrant d'ostéoporose et avec ce produit, nous pouvons nous passer de médicaments fabriqués chimiquement qui ont le plus latéral symptômes comme les capsules.

Nous avons fait de nombreuses recherches, mais aucun produit similaire à notre produit n'a été trouvé sauf pour les capsules d3 industrielles où notre invention est complètement différente de tous les médicaments fabriqués car il est 100% naturel et facile à fabriquer économiquement et accessible à tous les segments de société.

Après avoir étudié en profondeur le marché algérien et international et mené plusieurs recherches en ligne, nous n'avons suivi aucun produit de notre produit. Nous avons décidé d'être les races dans ce domaine en faisant des gâteaux riches en vitamine D3 et de nombreux composés énergétiques tels que le calcium, le fer et beaucoup d'autres vitamines comme A, C, protéines et c'est pour le corps humain pour prévenir les maladies et construire un corps sain et intégré.

List of abbreviation

- D3: iodthyrnine dioses type 3
- D2: iodthyrnine dioses type 2
- UVB: UBV rays are ultraviolet rays with medium wavelengthsRda: République démocratique allemande
- (25-OH-D) : Le Calcifédiol, également connu sous le nom de calcidiol, 25-hydroxycholécalciférol, ou 25-hydroxyvitamine D_3, est une forme de vitamine D produite dans le foie par hydroxylation de la vitamine D_3 par l'enzyme vitamine D 25-hydroxylase.
- PTH : parathyroid hormone
- SLE :systemic lupus erythematous
- Nmol/l: nanomoles per litre , a mol is an amount of a substance that containe a large mumber

  L: litre measure fluid volume .it is a littre bigger than a quart.
- Ng/ml: nanogramme per litre

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Introduction

Nowadays, food industry is searching for solutions to reduce the levels of certain ingredients in their products, such as salt, fats and sugars, in order to produce healthier foods that meet the consumers' expectations. High sucrose levels are associated with health problems such caries, obesity, type II diabetes, high blood cholesterol and coronary diseases. During the baking process complex, biochemical and physicochemical reactions occur, which can affect the quality and safety of the biscuits.

Due to the growing consumer demands for convenient food, biscuits represent one of the fast-growing segments of bakery products. Ready to eat, unique taste, readily available, long shelf life, and good eating quality are characteristics which enhance its consumption (Adeola & Ohizua, 2018). However, most biscuits are made with butter and margarine which contain high amount of fats, and lower amount of vitamin sincerely VITAMIN D with saturated and trans fatty acids. They also contain good quantities of sugar and salt which might be the leading causes of health problems such as diabetes, hypertension, obesity, and cardiovascular diseases (Lloyd-Williams et al., 2009).

As the prevalence of these non communicable diseases increases worldwide, consumers become worried about their health and so demand snacks which can provide health benefits with a good nutritional value. Wheat flour is a basic ingredient in the preparation of biscuits (Andualem et al., 2016). However, its conditions of cultivation associated to the high cost of its importation as well as the phenomenon of gluten intolerance have led to the development of several researches in order to find alternatives to wheat.

Nowadays, researches are directed towards the use of local raw materials due to their composition which will improve the nutritional composition of biscuits and also to their great potential for improving the sustainability of food systems (Pasqualone et al., 2020). In this light, the promotion of the use of composite flours, which integrate flours derived from local crops (oats, cereals,), and the good source of oil such us cod liver oil enriched on vitamin and amino acid in bakery products such as biscuits is being encouraged.

Evidence about the diets of early humans has served as the foundation for numerous arguments in support of higher calcium and other nutrient intakes(Olaoye et al., 2006). Similarly, early humans' blood levels of 25(OH)D (calcidiol), a form of vitamin D, were undoubtedly far greater than what is now considered to be normal.

Vitamin D deficiency causes rickets in children and osteomalacia in children and adults.1 Rickets is characterized by a failure or delay in endochondral ossification at the growth

plates of long bones that, in children old enough to stand, results in characteristic bone deformities of the lower limbs. Osteomalacia is defective mineralization of osteoid on the trabecular and cortical surfaces of bone and is associated with widened osteoid seams and the presence of Looser zones. Both conditions may be associated with pain, hypocalcemic fits, and muscle weakness in the limbs, heart, and respiratory systems. Low vitamin D status, above that associated with clinical deficiency, has also been linked with an increased risk of other diseases, most notably osteoporosis, cardiovascular disease, diabetes, some cancers, and infectious diseases such as tuberculosis

Parfitt et al. (Vitali et al., 2009)state that "vitamin D deficiency implies the existence of an anatomic, physiological, or biochemical abnormality that can be corrected by the administration of vitamin D in nonpharmacological doses." PTH concentrations become negligible when 25(OH)D

In this context the objective of our work was to produce a food compliment; biscuit enriched with vitamin D Cod liver oil and oats flower.

Our work was composed of two main parts:

- Bibliography section which includes general information on vitamin D
- The second part which consist of materiel and methods and results and discussion

Chapter I: General information about *Cod liver oil*

Introduction

Nutritional research into health and nutritional benefits continues. Cod liver oil is a dietary supplement extracted from cod which contains large quantities of essential vitamins (D, A and omega-3 fatty acids); this means that the body cannot synthesize them on its own, so the only way to obtain these essential nutrients is through the diet ,or by consuming supplements containing them. It is available in liquid and capsule form, and recommended doses vary according to the manufacturer, but generally exceed 30 millilitres per day.

I.1.The biological activities of CLO

Today, the considerable interest to consume several foods having the biological activities to treat and/ or to prevent certain diseases has appeared. Numerous studies have been explored to correlate the certain foods with its beneficial effects to human health, currently, known as functional food (Rohman & Che Man, 2012).

I.1.1. CLO and coronary heart disease

For many years, fish oils including CLO is associated with good health effects. The American Heart Association recommend to patients with coronary heart disease, the consumption of 1 g of fish oil per day, preferably by eating fish. The epidemiological studies also revealed that there is an inverse relationship between high fish oil consumption and the low mortality following coronary heart disease, possibly through the changes in prostaglandin metabolism (Blakytny & Jude, 2006)

I.2. CLO and anticancer Daily

use of CLO was associated with reduced risk of death in patients with solid tumors (Skeie et al., 2009). The supplementation of CLO daily for at least a year in patients with solid tumors such as breast, colon, lung, but not blood cancers had a death risk of 33% less than those who used CLO less frequently, while patients with lung cancer, the mortality reduction was 44%. Vitamin D and omega-3 fatty acids in CLO contributed to lung cancer survival.

3.3. Antidiabetic activity of CLO

CLO was also reported to be correlated with the lower risk of diabetes mellitus insulin-dependent (Type I). These protective effects were caused by vitamin D or the omega-3 fatty acids of EPA and DHA present in CLO (Louise Holmen Terkelsen, 2000) reported that CLO with dose 25% (wt/wt) in ointment preparation can significantly accelerate both the epithelial and the vascular component of healing compared with saline.

I. 3. Benefits of cod liver oil for children

A study published in the Annals of Otology, Rhinology & Laryngology in 2002 showed that the daily intake of a teaspoon of cod liver oil, in addition to selenium-containing vitamin and mineral supplements by children taking part in the study, can help reduce the risk of otitis media and reduce their need for antibiotics. To find out more about the benefits of cod liver oil for children, please refer to the article cod liver for children.

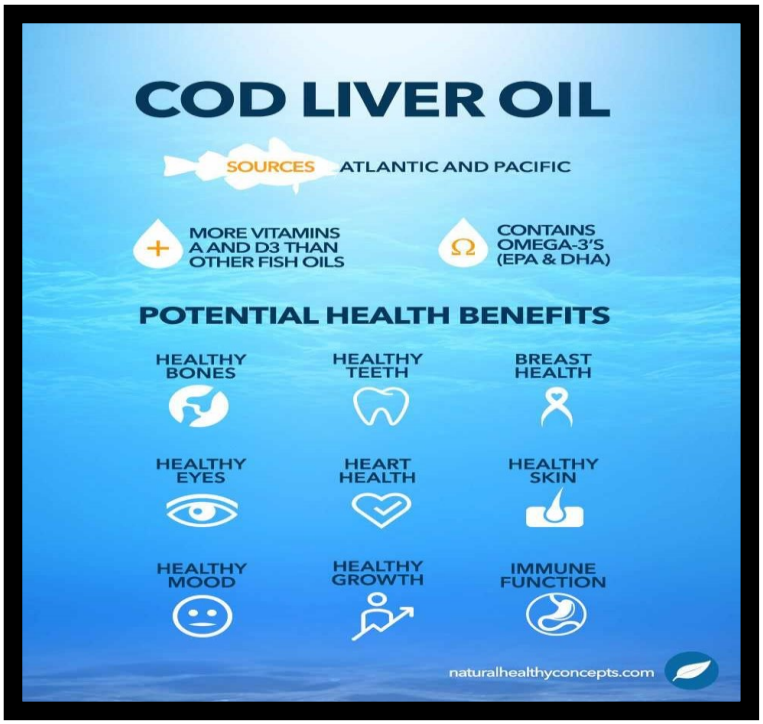


Figure 1 : Benefits of cod liver oil for children

I.4. Undesirable effects

Safe cod liver oil, or cod liver oil, is safe mainly for adults and children. However, it may cause certain side effects, such as: belching, heartburn, diarrhea, nausea, bad breath. These effects

can be alleviated by taking cod liver oil with meals, but it is potentially dangerous to take cod liver oil in high doses, as in this case it can cause bleeding due to its anticoagulant properties, and this can also lead to increased levels of vitamin A and vitamin D in the body,

- It should be noted that it is potentially safe for pregnant and breastfeeding women to take cod liver oil in quantities that do not provide more than their body's requirements of vitamin D, vitamin A, while it is not safe to take it in larger quantities; because taking dietary supplements that contain Vitamin A during pregnancy can be hazardous to the fetus and should only be used after consulting a doctor

I.5. Physico-chemical properties of Cod liver oil

Physico-chemical properties of edible fats and oils are important for characterization of fats and oils. Different physical and chemical parameters of edible fats and oils can be exploited for the monitoring the quality of oils .These physicochemical parameters include iodine value, saponification value, viscosity, density, and peroxide value, anisidine value, oxidation products, and volatile compounds (Kholmanov et al., 2012). In addition, these physical values are also used for identification of fats and oils from adulteration practices. The fatty acid (FA) composition of CLO along with physical and chemical properties is compiled in Table 1.

Table 1: Physico-chemical properties of Cod liver oil

Physical properties*	Value
Iodine value (mg I_2/g)	162 (159 – 166)
Slip point (°C)	< 10
Saponification value (mg KOH/g)	186 (185 – 187)
Refractive index	618 (615 – 621)
Fatty acid scomposition	Level (%)
Saturated fatty acid (SFA)	
C14: 0	3.8
C16: 0	9.4
C18: 0	2.1
ΣSFA	15.3
Monounsaturated fatty acid (MUFA)	
C16:1n-7	7.9
C18: 1n-9	17.1
C18: 1n-7	4.2
C20: 1n-9	10.9
C22: 1n-11	5.3
C22: 1n-9	0.5
ΣMUFA	45.9
Polyunsaturated fatty acid (PUFA)	
C18: 2n-6	1.8
C18: 3n-3	1.0
C18: 4n-3	3
C20: 5n-3	10.1
C22: 5n-3	1.2
C22: 6n-3	11.9
ΣPUFA	27.2

I.5.1.cod liver oil as vitamin D sources

Vitamin D is essential for the maintenance of good health. Its sources can be skin production and diet intake. Most humans depend on sunlight exposure (UVB 290–315 nm) to satisfy their requirements for vitamin D. Solar ultraviolet B photons are absorbed by the skin, leading to transformation of 7-dehydrocholesterol into vitamin D3 (cholecalciferol). Season, latitude, time of day, skin pigmentation, aging, sunscreen use, all influence the cutaneous production of vitamin D3. Vitamin D deficiency not only causes rickets among children but also precipitates and exacerbates osteoporosis among adults and causes the painful bone disease osteomalacia. Vitamin D deficiency has been associated with increased risk for other morbidities such as cardiovascular disease, type 1 and type 2 diabetes mellitus and cancer, especially of the colon and prostate. .(Holick, 2007; Nelson & Cox, 2005; Norman, 2008)

1. Chemical structure and nomenclature

Vitamin D is a fat-soluble vitamin that exists in several forms, but the most important form for the body is vitamin D3 (cholecalciferol).The chemical structure of vitamin D3 consists of a tetracyclic steroid nucleus, which is derived from cholesterol. The steroid ring is made up of four

fused rings, called A, B, C and D. Ring A is a six-membered ring, while rings B, C and D are five-membered rings. Vitamin D3 also has a hydrocarbon side chain attached to the D ring. This side chain contains three methyl groups (-CH3) and one hydroxyl group (-OH).

In summary, vitamin D3 is a fat-soluble vitamin that has a tetracyclic steroid ring derived from cholesterol, as well as a hydrocarbon side chain containing three methyl groups and one hydroxyl group. Its systematic nomenclature is 9,10-secocholesta-5,7,10(19)-triene-3β,25-diol.

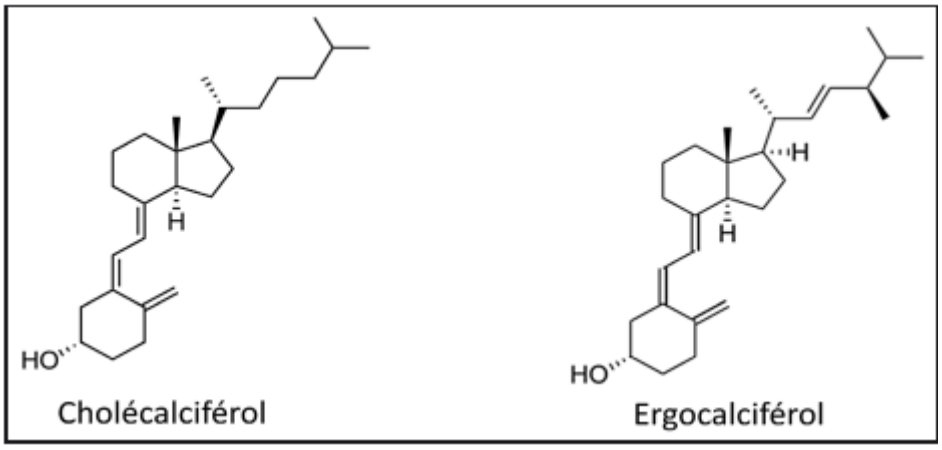


Figure 2: : Chemical structure and nomenclature

2.Source of vitamin D

The major source of vitamin D for most humans has always been exposure to sunlight;4,11 however, oily fish, including salmon,3 is an excellent source of vitamin D3 that typically contains 500–1000 IU/3.5-ounce serving. However, salmon farmed in the United States receive very little vitamin D in their pelleted diet and have been found to contain just 10–25% of the vitamin D3 content found in wild-caught salmon.12 Foods fortified with vitamin D in the United States include milk, orange juice, some breads, margarines, cheeses, and yogurts. In Europe, Sweden, and Finland, milk is fortified with vitamin D3, but most other European countries still forbid the fortification of dairy products with vitamin D. Other sources include margarine and some cereals. Farmed salmon from Norway Figure 1 Ultraviolet absorption spectra for (A) previtamin D3, (B) tachysterol, (C) provitamin D3 (7-dehydrocholesterol), (D) lumisterol, (E) DNA, and (F) albumin. from are fed fish oil, and thus contain a similar amount of vitamin D3 in their flesh as wild-caught salmon. Sundried

mushrooms are a natural source of vitamin D2. A wide variety of vitamin D supplements is available worldwide.2,4,

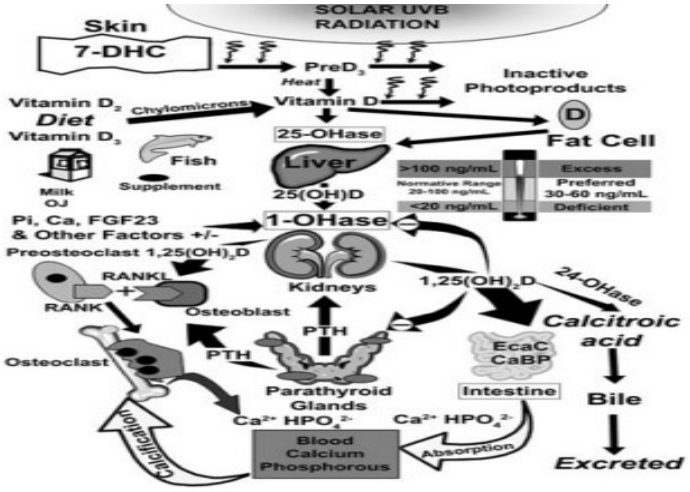


Figure 3: SOURCES OF VITAMIN D

Schematic representation of the synthesis and metabolism of vitamin D regulating calcium, phosphorus, and bone metabolism

3.Vitamin D Metabolism

The three main steps in vitamin D metabolism, 25-hydroxylation, 1a-hydroxylation, and 24-hydroxylation are all performed by cytochrome P450 mixed-function oxidases (CYPs). These enzymes are located either in the endoplasmic reticulum (ER) (e.g., CYP2R1) or in the mitochondria (e.g., CYP27A1, CYP27B1, and CYP24A1). The electron donor for the ER enzymes is the reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent P450 reductase. The electron donor chain for the mitochondrial enzymes is comprised of ferredoxin and ferredoxin reductase.

These include 12 helices (A–L) and loops and a common prosthetic group, namely the iron-containing protoporphyrin IX (heme) linked to the thiolate of cysteine. The I helix runs through the center of the enzyme above the heme where a thr(ser) and asp(glu) pair is essential for catalytic activity(Matsumoto et al., 2012). CYP2R1, like other microsomal CYPs, contains two extra helices

that appear to form a substrate channel in the bilayer of the ER (Sugimoto and Shiro, 2012). The B0 helix serves as a gate, closing on substrate binding. Whether a similar substrate channel exists for the mitochondrial CYPs is not clear.

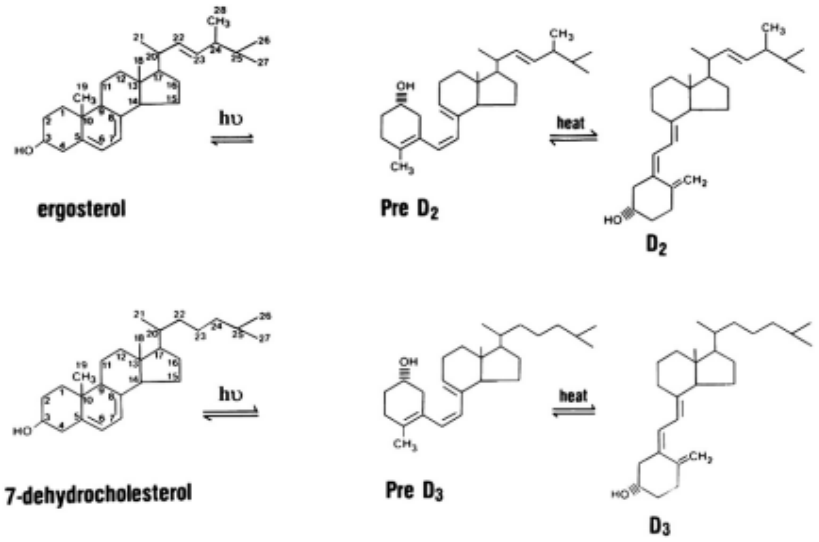


Figure 4: The structures of various vitamin D analogs currently in use clinically

All genomic actions of 1,25(OH)2D are mediated by the VDR. VDR is a transcription factor and member of the steroid hormone nuclear receptor family. It is comprised of three domains: the N-terminal DNA binding domain with two zinc fingers that bind to the grooves of the DNA at discrete sites (VDREs), the C-terminal ligand binding domain, and the hinge region binding these two domains together. The ligand binding domain structure has been solved by x-ray crystallography (Rochel et al., 2000). It is comprised of 12 helices.

These complexes can be both gene and cell specific, enabling the selectivity of 1,25(OH)2D action from cell type to cell type. These complexes include a subunit that directly binds to the VDR generally through an LXXLL motif along with a number of subunits that contain enzyme activity such as histone acetyl transferases (coactivators such as the SRC family) or deacetylases (corepressors such as SMRT and NCoR), methyl transferases and demethylases, ATPase-containing nucleosomal-remodeling activity (SWI/SNF), and links to RNA polymerase II (Mediator complex).

Vitamin D deficiency is also common in children and in young and middle-aged adults. In one study, 36% of otherwise healthy young adults aged 18 to 29 years had a 25(OH)D level of <20 ng/mL at the end of winter, despite the fact that they often took a multivitamin, drank at least one glass of milk a day that was fortified with vitamin D, and ate fish at least once a week.38 Another study showed that 48% of preadolescent white girls living in the US state of Maine had a 25(OH)D level of <20 ng/mL at the end of winter.39 At the end of summer, 17% still had a 25(OH)D level of <20 ng/mL; many of these girls attended summer camp, but they often wore sun protection or avoided direct sun exposure

4. Vitamin D deficiency

It is now regarded by most experts to be a 25(OH)D level <20 ng/mL (50 nmol/L).4,11,13,26–30 Since the efficiency of intestinal calcium absorption is enhanced by as much as 65% when osteoporotic women raised their blood levels of 25(OH)D to >30 ng/mL (75 nmol/L)31 and the fact that parathyroid hormone levels begin to level off at their nadir, between 30 and 40 ng/mL28,32 (Figure 6), most experts agree that a 25(OH)D level >30 ng/mL is considered to represent sufficient vitamin D while levels between 21 and 29 ng/mL are insufficient.

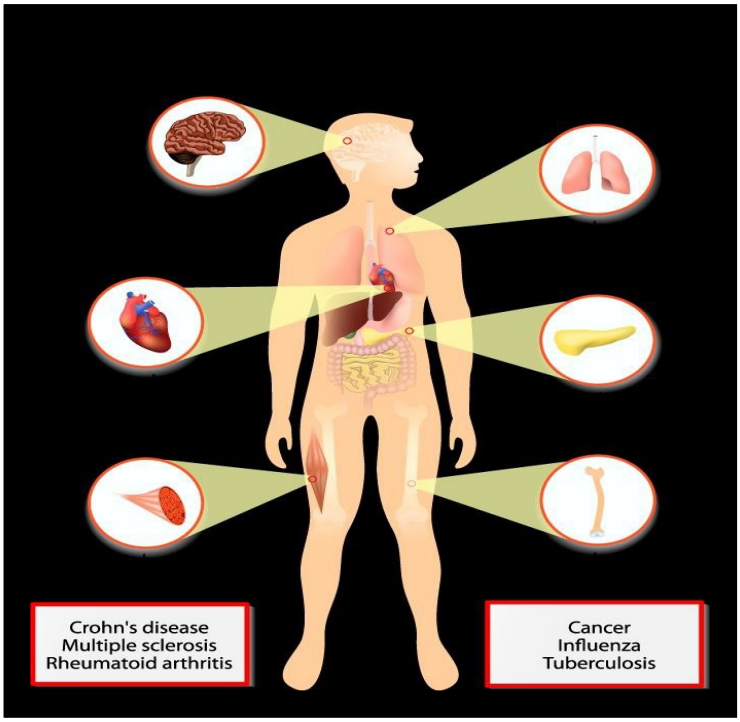


Figure 5: Vitamin D deficiency

5. Clinical Applications

The literature assessing the relationship of vitamin D adequacy to human disease is vast, and attempts to summarize it in a few short paragraphs is not feasible. However, there are several points that can be made about some of the clinical applications that have received the most study.

- **The Skeleton**

There is little controversy that adequate vitamin D is necessary to prevent rickets and osteomalacia. More controversy exists with respect to the role of vitamin D in the prevention of osteoporosis and fractures . However, a meta-analysis of a number of randomized controlled trials demonstrated a positive dose-response relationship between vitamin D supplementation and fracture prevention (Bischoff-Ferrari et al., 2009)

- **The PTG**

The inverse relationship between circulating 25OHD levels (but not 1,25(OH)2D levels) and PTH levels is well established, but the degree of variability in the population with respect to

this relationship is large. Nevertheless, PTH levels are a useful marker for vitamin D sufficiency, and maintaining adequate levels of 25OHD in the blood will reduce the risk for PTG hyperplasia and elevated PTH secretion with its potential deleterious effects on bone. The PTG expresses both the VDR and CYP27B1. The PTH gene contains a negative response element for 1,25(OH)2D /VDR (Demay et al., 1992)

- **The Skin**

The use of the 1,25(OH)2D analogs calcipotriol and maxacalcitol for the treatment of the hyperproliferative skin disease psoriasis represents another approved clinical application outside of the skeleton for vitamin D and its analogs. Psoriasis is a disorder with hyperproliferation and decreased or abnormal differentiation driven by an abnormal immunologic component. The successful use of 1,25(OH)2D and several of its analogs is likely due to their ability to inhibit the proliferation, stimulate the differentiation, and suppress the immune activity associated with this disease (Bikle, 2012b)

Obesity, Diabetes Mellitus, and Metabolic

- Syndrome 25OHD levels are typically lower in obese individuals who are more likely to develop diabetes mellitus and the metabolic syndrome. Adipocytes express the VDR, and 1,25(OH)2D promotes increased lipogenesis and decreased lipolysis The pancreatic b cell expresses the VDR, and 1,25(OH)2D promotes insulin secretion . Moreover, vitamin D deficiency is associated with insulin resistance (Kayaniyil et al., 2010).
- Cancer The data from animal and cell culture studies are very promising that 1,25(OH)2D or its analogs can prevent cancer development or retard its progress/metastasis once developed (Bikle, 2004). The mechanisms by which 1,25(OH)2D can suppress tumor development are numerous and in many cases cell specific. These include inhibition of proliferation by blocking elements of the cell cycle or interference with signaling by

growth factors, inducing apoptosis, stimulation of DNA damage repair, prevention of tumor angiogenesis, and inhibition of metastasis

- Cardiovascular Disease The VDR and CYP27B1 are expressed in the heart, both in the myocytes and in the fibroblasts . 1,25(OH)2D and its analogs suppress markers of cardiac hypertrophy, and deletion of the VDR specifically from the heart results in hypertrophy (Chen et al.,). VDR and CYP27B1 null mice are also hypertensive with increased production of renin from kidneys and heart resulting in increased circulating angiotensin II levels

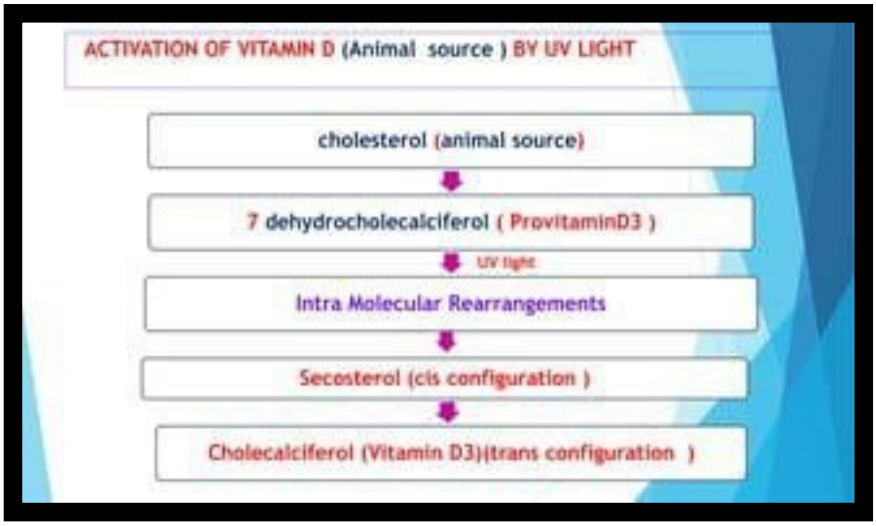


Figure 6: Clinical Applications

6. Storage of vitamin D3

Vitamin D is a fat-soluble vitamin that is stored in adipose tissue (fat) and muscles. It is produced by the skin when exposed to sunlight and can also be obtained from dietary sources such as oily fish, eggs and fortified dairy products.

The storage of vitamin D in the body depends on a number of factors, including the amount of vitamin D produced or consumed, exposure to sunlight, the amount of body fat and the body's ability to absorb and use vitamin D.

In general, people who are regularly exposed to sunlight and have a balanced diet of vitamin D tend to have higher levels of vitamin D and greater storage in their bodies. However, people who have limited exposure to sunlight or who are deficient in vitamin D may have lower levels and reduced storage.

It is important to note that vitamin D is an essential vitamin for healthy bones and teeth, as well as for the immune system and muscle function. It is therefore advisable to maintain adequate levels.(Cashman et al., 2016; Ellison & Moran, 2021; A Catherine Ross et al., 2011)

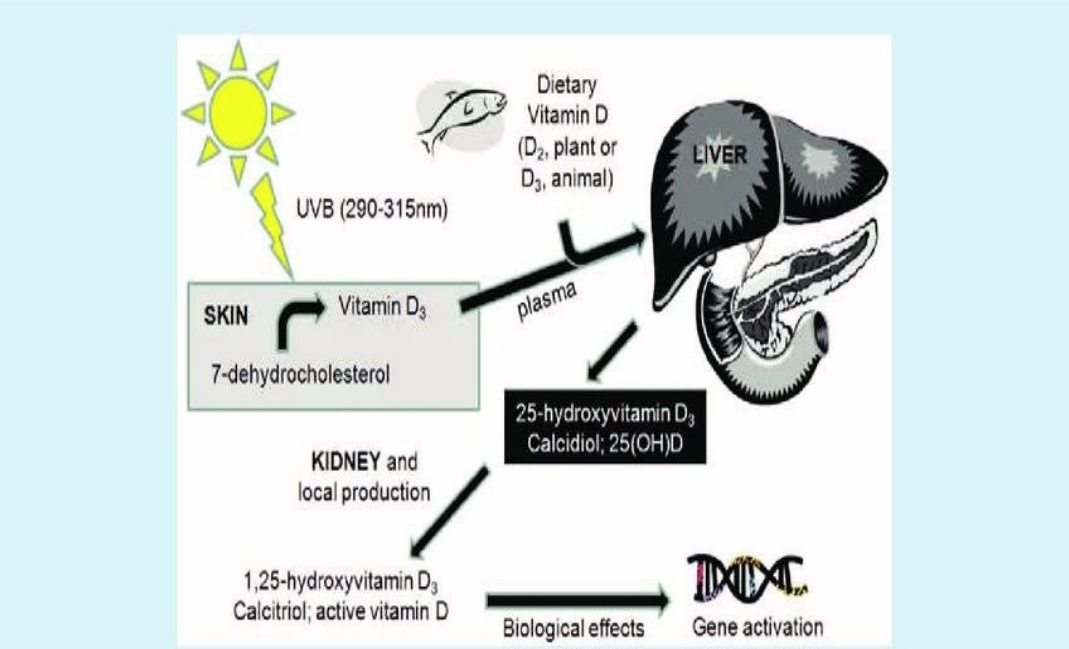


Figure 7: Storage of vitamin D3

7. Regulatory mechanism

Vitamin D is a fat-soluble vitamin that plays an important role in regulating calcium and phosphorus metabolism in the body. It is produced in the skin when exposed to sunlight, but can also be obtained from dietary sources such as oily fish, eggs and dairy products.

The mechanism for regulating vitamin D is complex and involves several organs and hormones. The main regulator of vitamin D is parathyroid hormone (PTH), produced by the

parathyroid glands in the neck. When calcium levels in the blood are low, PTH is released, which stimulates the production of vitamin D in the kidneys.

In short, the vitamin D regulatory mechanism involves several organs and hormones, with PTH as the main regulator. Active vitamin D stimulates the absorption of calcium and phosphorus in the intestines and the release of calcium in the bones, which maintains the balance of calcium and phosphorus metabolism in the body.

Vitamin D is then converted into its active form, 1,25-dihydroxyvitamin D, in the kidneys. This active form of vitamin D acts on the intestines to increase the absorption of calcium and phosphorus into the blood. It also acts on the bones to stimulate the release of calcium into the blood.

The vitamin D regulatory mechanism is also influenced by other hormones, such as growth hormone, insulin and sex hormones. Vitamin D levels can also be affected by factors such as age, skin colour, sun exposure and diet(Christakos et al., 2016; Venter et al., 2001)

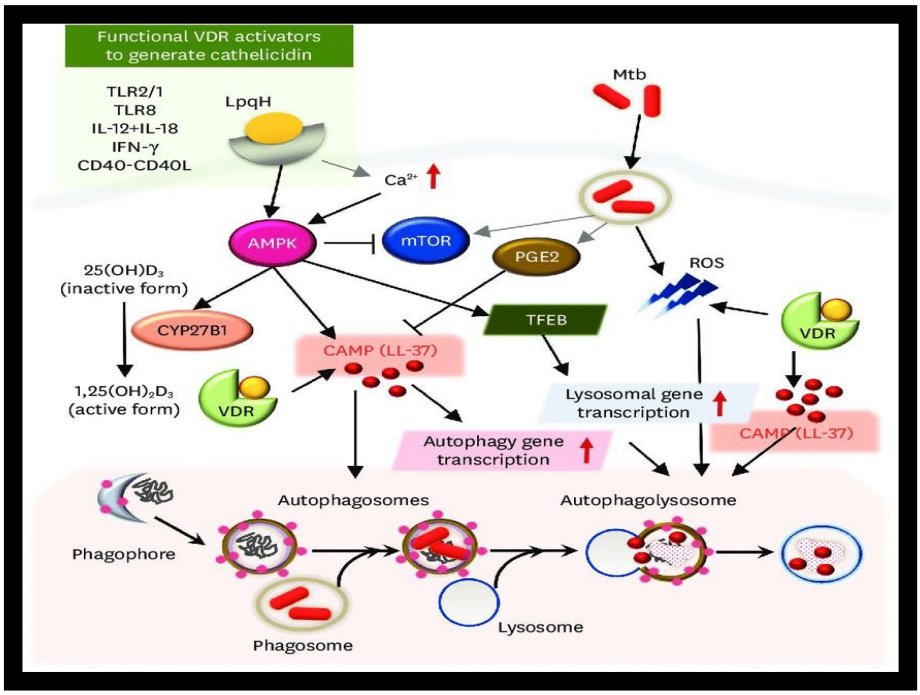


Figure 8: Regulatory mechanism

Chapter II

Technology of biscuit

Introduction

The consumption of cereal snack foods such as biscuits, cookies, wafers and short bread has become very popular in Sri Lanka especially among children. Among these biscuits possess several attractive features including wider consumption base, relatively long shelf-life and good eating quality. Long shelf-life of biscuits makes the possibility of large scale production and distribution. Good eating quality makes biscuits attractive for protein fortification and nutritional improvements, particularly in children feeding programmes, for the elderly and low income groups. Enrichment of cereal-based foods with other protein sources such as oil seeds and legumes has received considerable attention(Ayo & Olawale, 2003; Olaoye et al., 2006). This is because of oil seeds and legume proteins are high in lysine, an essential limiting amino acid in most cereals.

II.1. Biscuit definition

Biscuits are dry, crunchy food products, usually sweet, that can be eaten as a snack, dessert or with a drink. They are made from a mixture of flour, sugar, fat, eggs and yeast or bicarbonateation, dessert or snack. Biscuits are made from a dough of flour, sugar, fat (such as butter or oil), eggs and other optional ingredients such as dried fruit, nuts, chocolate, spices or flavourings. They come in different types, shapes and sizes, such as shortbread, cookies, macaroons, sandwich biscuits, galettes, madeleines, crunchy biscuits, speculoos, etc. Biscuits are widely consumed throughout the world and are among the most popular bakery products.

II.2. Classification of biscuits

1.Savoury biscuits

- Crackers: thin, crispy biscuits flavoured with cheese, herbs, paprika, etc.

- Savoury shortbread: biscuits made with flour, butter and salt, often flavoured with herbs or spices.

- Gressins: long, thin biscuits with pepper, etc.

- Sablés salés: crumbly biscuits often flavoured with cheese and spices.

- Grissini: long, thin, crunchy Italian baguette-shaped biscuits, often served with savoury dishes.

- Aperitif biscuits: various biscuits in the form of salty snacks such as crisps, pretzels, mini pizzas,etc.

2. Sweet biscuits

- Shortbread: crumbly, crumbly biscuits, often flavoured with vanilla or lemon.

- Cookies: soft, thick biscuits with chocolate chips, nuts, dried fruit, etc.

- Petits fours: biscuits made in several stages using ingredients such as marzipan, jam, cream, chocolate, etc.

- Macaroons: round, coloured biscuits filled with ganache, jam or butter, often decorated with patterns or sparkles.

These categories are not exhaustive and there are many other types of biscuit made all over the world.

II.3. General information about Madeleine

Madeleine is a traditional French cake that is often served with a cup of tea or coffee. Although there are many different recipes, the classic madeleine is generally made with eggs, sugar, flour, yeast, butter and lemon zest. The shape characterises different varieties of madeleine, the classic version is usually made from a light, airy pastry, flavoured with lemon zest and baked in a shell-shaped mould.

Madeleine originated in the Lorraine region of eastern France, where it was created in the 18th century. According to legend, the cake was first invented by a cook called Madeleine, who worked for the Duke of Lorraine. The traditional recipe has since been passed down from generation to generation in many regions of France and has become a symbol of French cuisine.

Today, madeleine is available in many flavour variations, including chocolate, vanilla, pistachio and coconut, among others. It is also popular as an artisanal product and is often sold in bakeries and patisseries.

II.3.1. Main ingredients and their effects

- **Flour**

Is a basic food ingredient used in many kitchens around the world. It is generally made by grinding cereals such as wheat, rye, barley, maize, rice or sorghum. Wheat flour is most commonly obtained by grinding cereal grains such as wheat, rice, maize, barley, oats or rye. Wheat flour is the

most commonly used flour and is available in different varieties such as all-purpose flour, whole wheat flour, white flour and spelt flour. Flour is used to make bread, pasta, biscuits, cakes, muffins, pancakes and much more. It is an important source of complex carbohydrates, fibre, B vitamins and minerals such as iron and zinc.

- **Fat**

There is a relationship between fat and vitamin D3 because vitamin D3 is a fat-soluble vitamin, which means that it dissolves in fats and oils. Consequently, a diet rich in fat can help to increase the absorption and storage of vitamin D3 in the body.

However, this does not mean that we should consume excess fatty products to increase vitamin D3 absorption. A balanced diet containing foods rich in vitamin D3, such as oily fish, milk and eggs, is generally sufficient to meet the body's vitamin D3 requirements.

- **Sugar**

As an RN, I don't have access to the latest scientific research. However, as far as I know, there is no direct relationship between sugar and vitamin D3. Sugar is a simple carbohydrate that provides energy to the body, while vitamin D3 is a fat-soluble vitamin that plays an important role in regulating calcium and phosphate metabolism. However, excessive sugar consumption can lead to weight gain and insulin resistance, which are risk factors for various health problems such as osteoporosis and vitamin D deficiency. It is therefore important to eat a balanced diet to maintain an optimal level of vitamin D3 in the body.(Wacker & Holick, 2013a)

- **Water**

There is no direct relationship between water and vitamin D3. However, vitamin D3 is often called the "sunshine vitamin" because it is synthesised in the skin when it is exposed to sunlight. It is important to drink enough water to stay hydrated and to help the body metabolise and produce itamin D3. Vitamin D3 is soluble in fat, not water, but it can be transported in the blood by lipids.

Consequently, a balanced diet and regular exercise also help to maintain adequate levels of vitamin D3 in the body.(Haussler et al., 2013; A Catherine Ross et al., 2011; Wacker & Holick, 2013a)

- **Eggs**

Eggs are a natural source of vitamin D3. In fact, vitamin D3 is formed naturally in the bodies of hens when their skin is exposed to the sun's rays. As a result, the eggs laid by these hens contain a significant amount of vitamin D3. However, the amount of vitamin D3 in eggs can vary depending on the season, the environment in which the hens are reared and their diet. Eggs can therefore be an important source of vitamin D3 for people who do not receive enough of this vitamin through sun exposure or diet.

II. 3.2. Stages of manufacture

1. Prepare the ingredients: collect all the ingredients for the recipe. This could include flour, sugar, eggs, whale liver oil, baking powder, milk, grated lemon, oat flour, etc.

2. Mix the dry ingredients: Mix the flour, sugar and baking powder in a large bowl.

3. Add the wet ingredients: Add the eggs, oil, milk and grated lemon to the dry mixture. Stir until the dough is formed.

4. Heating oven: preheat the oven for the temperature recipe.

5. **Bake:** Bake the biscuits in the preheated oven for the time indicated in the recipe or until golden brown.

6. Cool the biscuits: Remove the biscuits from the oven and leave to cool on the cooling rack. Once cooled, store in an airtight bowl until ready to use.mme de fabrication de biscuit.

II.3.3. The sensory evaluation parameters:

The parameters used to assess the organoleptic quality of a food product can vary depending on the type of product and consumer preferences. However, here are a few criteria commonly used to assess the organoleptic quality of foods:

1. Appearance: the general appearance of the product, including its colour, size, shape, surface texture and shine.

2. Odour: the aroma of the product, which can be assessed before and after cooking or preparation.

3. Flavour: the taste of the product, which can be assessed in terms of sweetness, acidity, bitterness, saltiness, umami, etc.

4. Texture: the tactile sensation of the product in the mouth, including its hardness, crunchiness, softness, viscosity, mellowness, etc.

5. Consistency: the consistency of the product, which can be assessed in terms of density, firmness, viscosity, elasticity, etc.

6. Freshness: the state of freshness of the product, which can be assessed in terms of aroma, texture, colour and flavour.

7. Uniformity: the consistency of product quality, which can be assessed in terms of homogeneity of texture, shape, size and colour.

8. Palatability: the attractiveness of the product to the consumer, which can be assessed in terms of individual preferences, taste satisfaction and desire to consume.

These criteria can be used individually or in combination to assess the organoleptic quality of a food product. Evaluations can be carried out by formal or informal tasting panels, or by blind consumption tests to assess consumer preferences.(de Normalisation; Ghosh & Chattopadhyay, 2012; Lawless & Heymann, 2010).

Chapter III:

Materials and Methods

III. 1. Raw materials

All the basic ingredients Oats flour, Cod liver oil and eggs, were purchased at specialised and certified food markets.

III.2. Methodology

III.2.1. Development of Vitamin D enriched biscuits

The method described by Eyenga et al. was used to produce the biscuits (Madeleine) . Briefly, cod liver oil (witch replace the vegetable oil) was introduced into a clean bowl and creamed thoroughly with a stainless steel spatula until it becomes soft and smooth. Powdered sugar was added to give a homogeneous mixture. Eggs lemon peel were added follow-up with milk, the mixture was homogenized with the spatula the end oats flour were added to form a paste. and the mixture was stirred to obtain the final biscuit dough. The dough was then put into the biscuit mold, and the desired biscuit shape was formed on a tray lightly greased with margarine. The trays randomly filled with shaped dough were introduced into an oven set at 160°C and baked for 20–25 min. The light-brown baked biscuits obtained were removed and put in a large tray to cool to room temperature before packaging. the madelin-shaped biscuits were decorated with chocolate

III.2.2. Optimization of the Formulation of Rice Biscuits

1. Experimental Design

The response surface methodology through a mixture design was used to perform the experiments. The independent variables and their ranges of variation were oats flour 100–200 g, cod liver oil 1–5 UI, eggs10-35 g and mik 100-150g. The responses measured were Aroma colour (cooking) smell sweetness, acidity, bitterness, softness, mealy, aftertaste and overall acceptability of the biscuits.

2. Modelling

The independent variables were related to the assessed responses through a second-order polynomial model using the following equation.

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^{\,2} + \sum\sum_{i<j} \beta_{ij} X_i X_j + \varepsilon.$$

3. Validation of the Model

The indicators used for model validation were coefficient of determination (R^2), the adjusted , R^2 and the lack of fit.

4. Optimization Procedure

The optimization procedure was performed to obtain the optimal levels of factors leading to the desirable responses. In order to define the workable optimum conditions, the model equations were graphically computed to visualize, by means of contour plots, the relationship between the factors and responses. Optimization with multiple responses was achieved using the desirability function. The experimental and predicted values of the responses were compared using a -test in order to validate the adequacy of response surface models for predicting the optimum responses.

5. Sensory Analysis of Biscuits

Twenty-four hours following the production of biscuits, a sensory evaluation was carried out . The expert panel was made up of 30 students aged between 23 and 25, who had previously been trained to evaluate products similar to those to be tested. 14 of them were then selected on the basis of the trained panel's ability to perform the required sensory evaluation tasks, and its ability to reproduce the results of the sensory analysis. The naive panel was made up of fifty-two people in different age categories: from 20 to 66 years old.

A six-point scale was used to evaluate the biscuits from “0 = very low” to “5 = very high”. The targeted attributes are: texture (smooth, grainy), odor (milky, fruity), flavour/taste (sweet, sour, bitter), and after-taste persistence (persisting, milky, bitter). Thus, panel members are invited to rate

the six coded samples on a "one to seven point" rating scale according to the intensity of each attribute, then in the end each panel member is invited to give a score for his overall preference of the six products on a hedonic rating scale of "one to nine points"..The mean scores were analyzed using analysis of variance (ANOVA) method and difference separated using Turkey's test.

6. Nutritional Analysis

The nutritional composition of optimized madeleines was evaluated. The water content of the biscuit was determined by the ISO 1666 standard. The lipid content was evaluated by extraction with petroleum ether as a solvent to determine the amount of vitamin D. The ash content (mineral matter) was also evaluated

7. Statistical Analysis

All experiments were performed in triplicates and values were expressed as deviation. The data obtained was subjected to analysis of variance and regression analysis using JMP and EXCEL STAT). The Student -test was used to compare values and the statistical significance was set at it.

Chapter IV:

Results and discussion

IV. 1. Optimization of Biscuit Preparation

Table presents the experimental matrix of the mixture design including the different combination of independent variables and the values of the responses assessed.

Table 2: Optimization of Biscuit Preparation

RUN	OATS FLOUR	COD LIVER OIL	Eggs	Milk	Aroma	Color	Smell	Flavor Sweet
1	200	35	5	150	.	7	7	5
2	200	10	1	150	6	4	3	3
3	100	35	1	100	3	6	7	6
4	200	35	1	125	6	7	6	6
5	100	35	3	150	4	4	7	5
6	100	10	5	100	6	3	3	3
7	200	35	5	100	3	7	7	5
8	100	22.5	5	150	5	5	7	6
9	150	10	1	100	4	5	5	7
10	100	10	1	125	6.5	7	7	6
11	150	22.5	3	125	5	5	6	5
12	200	22.5	3	125	6	5	6	5
13	150	35	5	125	6.3	6	5	4
14	200	10	5	125	6	4	6	6
15	150	22.5	1	150	6	5.5	5.5	5
16	150	10	3	150	7	5,5	5.5	5

IV.1.1. Model Fitting

The results presented in Table 2 were exploited by applying the least-squares method and models were established. The following second-order polynomial equations explain the different responses measured as a function of the quantity of Oats flour, cold liver oil eggs and milk,

$$Y1 = - 9,97 + 0.1488\ X1 + 0,0078X2 + 0,3928X3 + 0,0919X4 + 0.0095X1X2 + 0:0025X1X3 + 0:0019X1X4 + 0.0013X2X3 + 0.0016X2X4 + 0:0029X3X4,$$

where Y1 = Aroma, , X1 = Oats flour, X2 = COLD LIVER OIL , X3 = Eggs, and X4 = Milk. The adequacy of these models to explain the variability in the different responses was evaluated by the coefficient of determination (R2), the adjusted R2, and the lack of fit. Table 3 presents the values

of the validation parameters. The second-order polynomial mathematical models were considered as valid taking into consideration the ranges recommended by Bas and Boyaci [21]

Table 3: Model Fitting

Parameter	**Df**	**Estimate**	**F**	**p**
Intercept		0,09199	0,0017*	0,0009*
Linear	1			
X1 Oats flour		0,0014		0,0017*
X2 Col liver oil	1	0,3992		0,0014*
X3 Eggs	1	0,0072		
X4Milk	1	0,0091		0,0001*
Interaction				
X1X2		0,0095		0,0020*
X1X3		0,0025		0,0003*
X1X4		0,0019		0,0003
X2X3		0,010		0,2507
X2X4		2,25		0,0036*
X3X4		0,01622		0,0036*
Pure error				
Lack of fit				
C. Total	14			0,0017*
R2	0,989			
Adj-R2	0,964			

IV.1.2. validation of model

The optimum conditions for formulation of the dough that maximize the different responses used to assess the quality of rice biscuits were determined based on contour plots **figure 10**. The

optimal conditions were illustrated in table the same conditions were optimised four all responses table 3 :

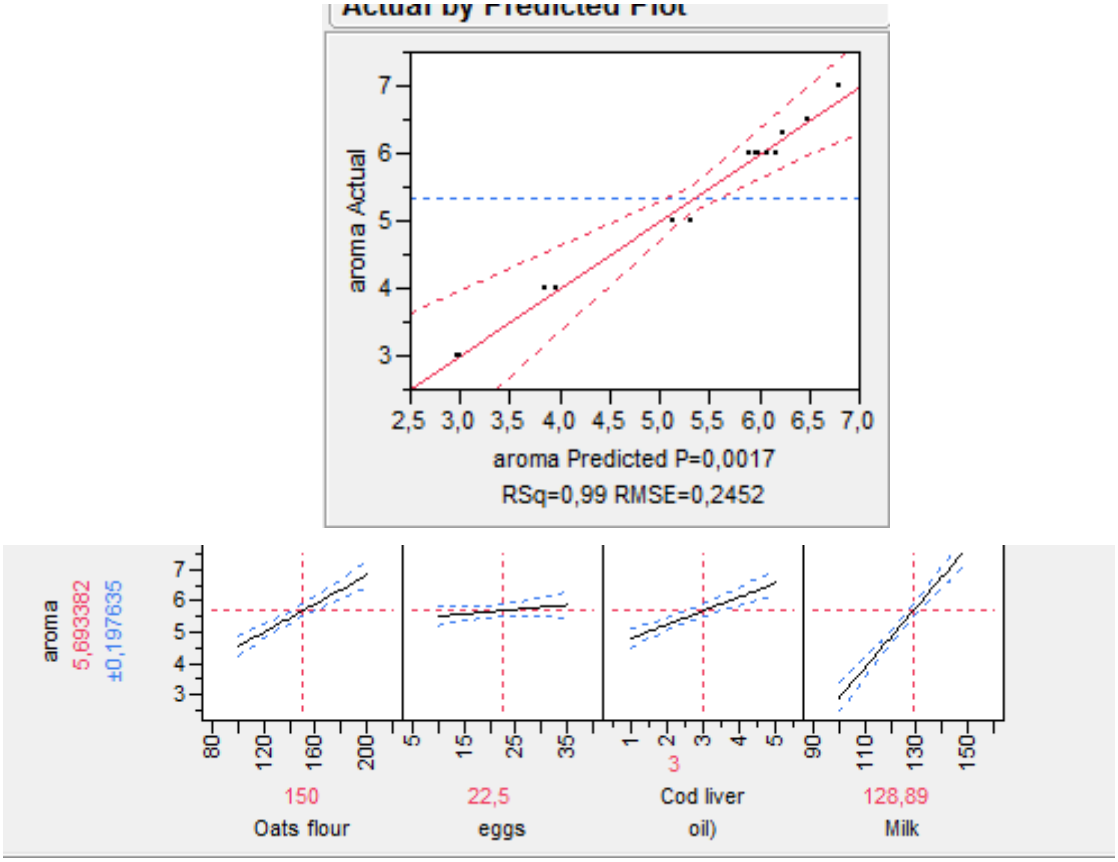


Figure 9: validation of model

Table 4: validation of model

Oast FLOUR (g)	COD LIVER OIL (UI)	Eggs (ml)	Milk (ml)	Aroma	Smell	Flavor Sweet
150	3	22. 5	128,89	5,69 ±0,19	5,83 ±0,03	5,18± 0,24

IV.2. Sensory evaluation

IV.2.1. Product characterisation

Product characterisation is used to describe the organoleptic characteristics of products based on the results of the expert panel. It also makes it possible to identify which descriptors best discriminate between products and which characteristics do not vary significantly between the samples analysed (Husson and Pages, 2009).

IV.2.2.Discriminating power by descriptor

The results of this test are illustrated in figure , which displays the descriptors ordered from the most discriminating to the least discriminating for the samples analysed.

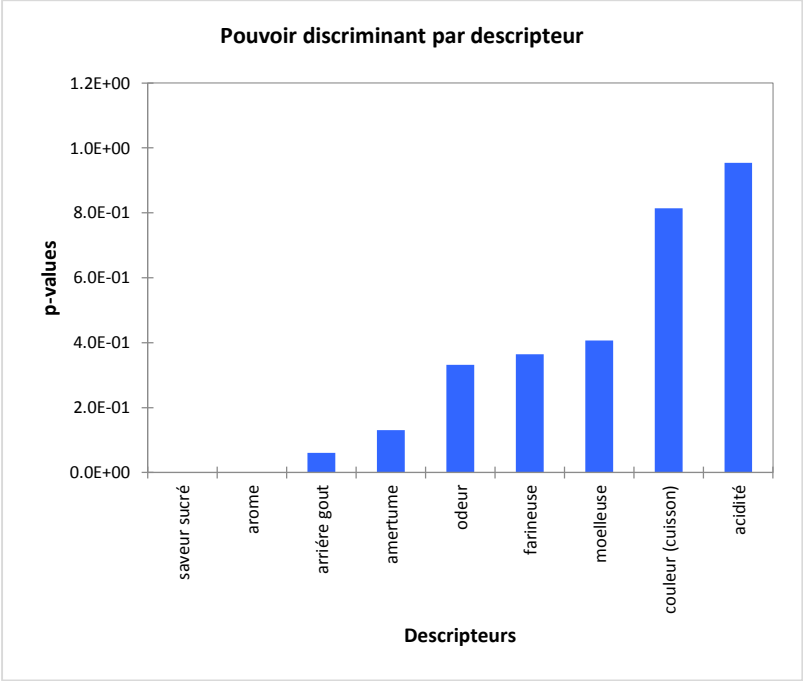


Figure 10:Discriminatory power by descriptor

The previous graph allows us to visualize that the sweet flavor and the aroma are the most discriminating descriptors, that is to say that these characteristics differ between the six cakes. On the other hand, the least discriminated descriptor is acidity and sweet color, which means that there is no difference in sweet taste for the four yogurts according to the judges.

Tableau 5: : Discriminating power by descriptor

Descriptors	Test values	p-values
sweet flavour	3.674	0.000
Aromas	3.005	0.001
After taste	1.554	0.060
Bitterness	1.123	0.131
Smell	0.434	0.332
Mealy	0.348	0.364
Soft	0.237	0.406
color (baking)	-0.893	0.814
Acidity	-1.684	0.954

IV.2.3. Model coefficients

In the figure 12 are displayed, for each descriptor and for each product, the coefficients of the selected model: The following figure allows you to see at a glance what defines the products (215.425.321 and 62).

The characteristics whose coefficient is significantly positive are given in blue, in red those whose coefficient is significantly negative and in white the characteristics whose coefficients are not significant.

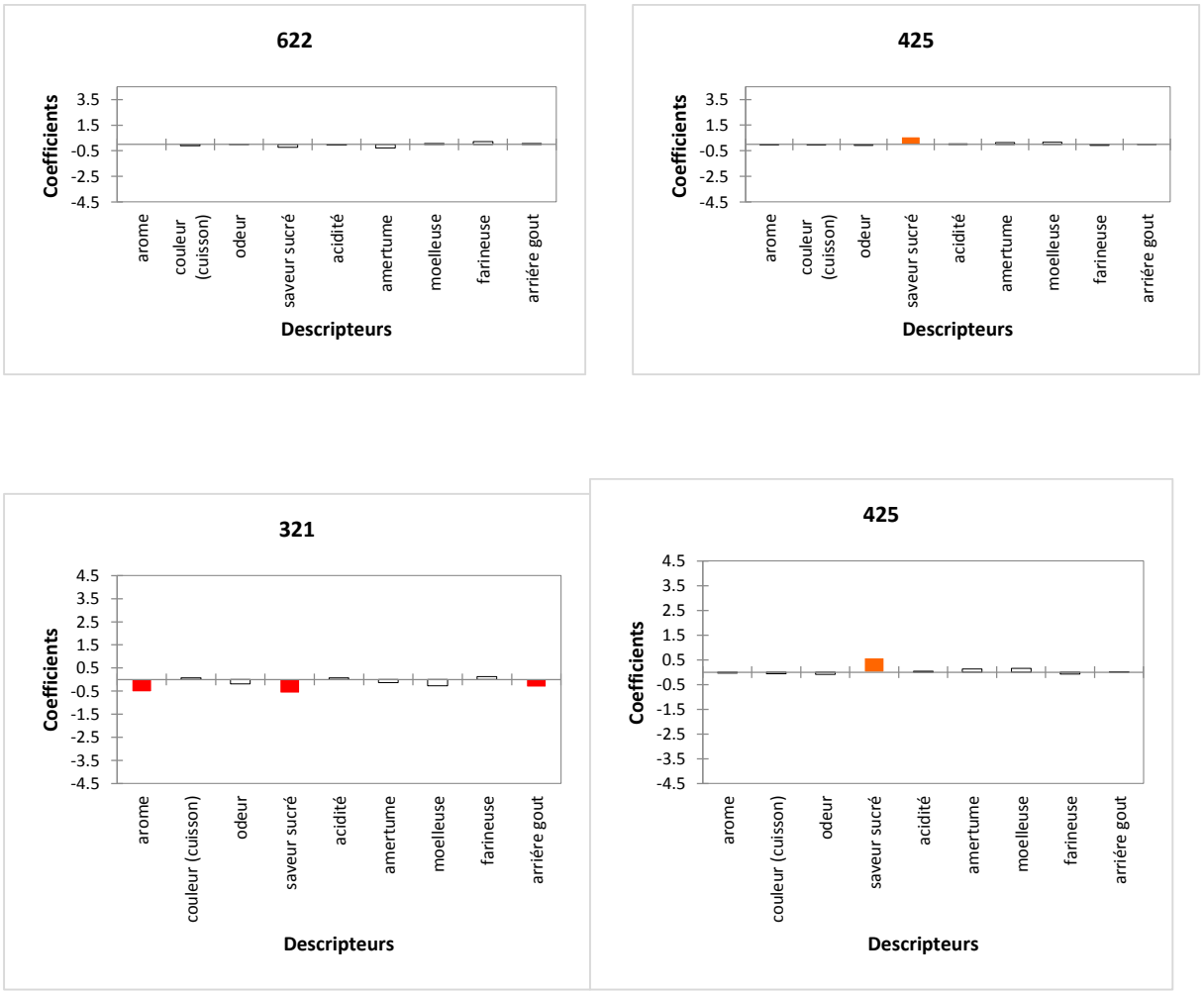


Figure11: Model coefficients of the Madeline samples and the control. Product-adjusted averages

In white, the characteristics of the product are displayed which have scores close to average, these characteristics are: smell, aroma, flavor, sweet flavor sour flavor and consistency, they have an average intensity according to the scores given by the judges experts.

622:.Characterized by a less intense odor but the other characteristics are close to the average of the scores that the judges gave.

215: characterized by an odor;, flavor (attributed aroma), sweet flavor, sour flavor, consistency and texture, close to the average of the scores that the judges gave with a very strong taste

421: characterized by a consistency and texture, close to the average of the scores that the judges gave with a very strong sweet flavor and taste

321: characterized by a very good smell, sweet flavor and absence of after-taste, consistency and texture, close to the average of the marks that the judges gave with a very strong sweet flavor

Conclusion

Conclusion

This work focuses on the design and formulation of a new food supplement enriched with vitamin D3 from cod liver oil. The idea behind this innovation is to meet the growing needs of people suffering from vitamin D3 deficiency, a global health problem that affects many people around the world.

For this a mixing plan was carried out with the optimal quantities of the ingredients of oat flour, cod liver oil, eggs, and milk being 150g, 3ml, 22.5 ml and 128.89 ml respectively which gave a rich biscuit in vitamin D with a good taste and mild flavour.

Finally, a sensory analysis for expert juries (people) was carried out and made it possible to study the acceptability and appreciation of the tasters towards the supplement developed. Our product enriched with vitamin D with cod liver oil was appreciated by tasters.

From all the results listed, we can deduce that the addition of cod liver oil in the formulation of the cake modified its physiochemical properties, its texture and its sensory properties.

In continuation of this work, it would be interesting to:

• Using an appropriate encapsulation technique can help preserve bioactive substances from environmental stresses.

• Optimization of added flour sugar concentrations

• formulation of other types of foods enriched with vitamin D

The project consists of marketing the innovative food supplement to the target audience and identifying appropriate distribution strategies to guarantee its availability and wide distribution.

Annexes

Annexes

Annexes 1

Exemple de fiche sensorielle

Nom: prénom: Age: sex : H F

Spécialité :

1	2	3	4	5	6	7

Code	4 65	7 77	3 25	66 2	17 2	322
Arome						
Couleur (cuisson)						
Odeur						
Saveur sucrée						
Acidité						
Amertume						

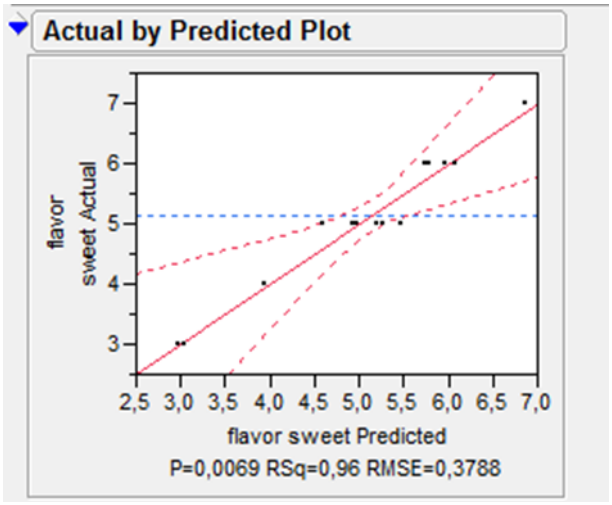
Texture :

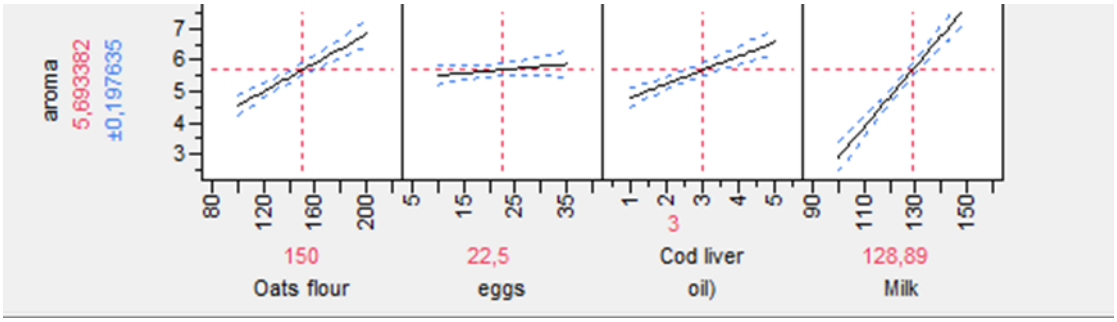
Texture Lisse						
Texture granileuses						
Arrière-gout						

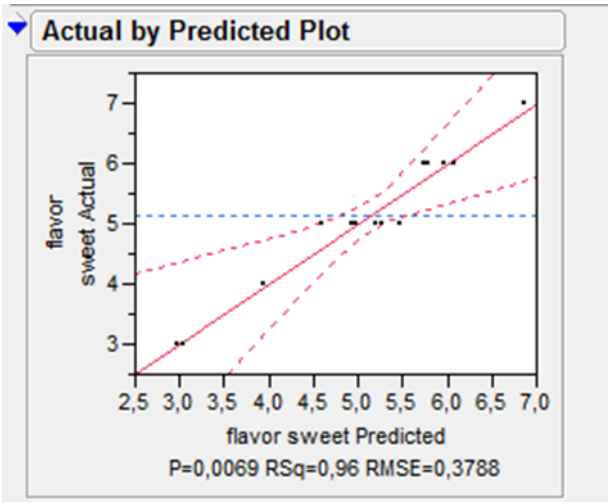
Veuillez indiquer votre satisfaction en cochant la case correspondante à l'intensité de votre plaisir.

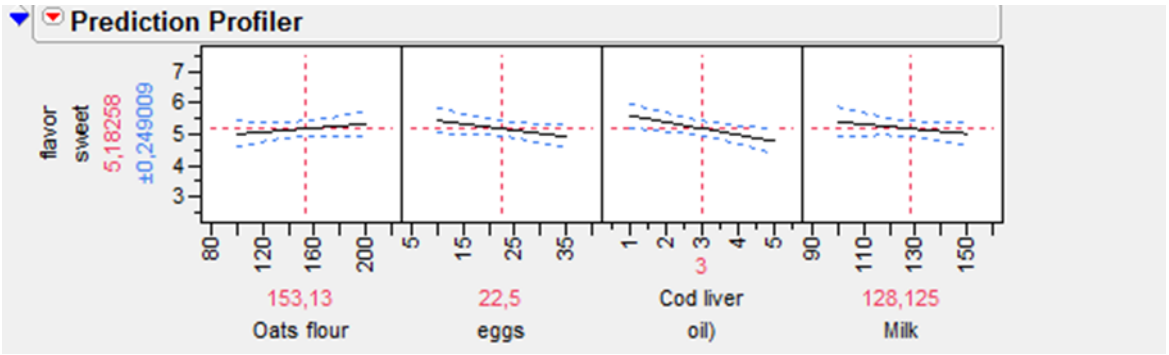
1 2 3 4 5 6 7 8 9

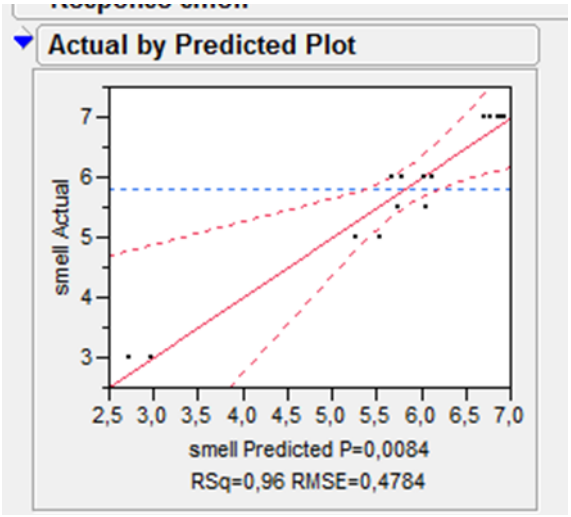
Annexes 2

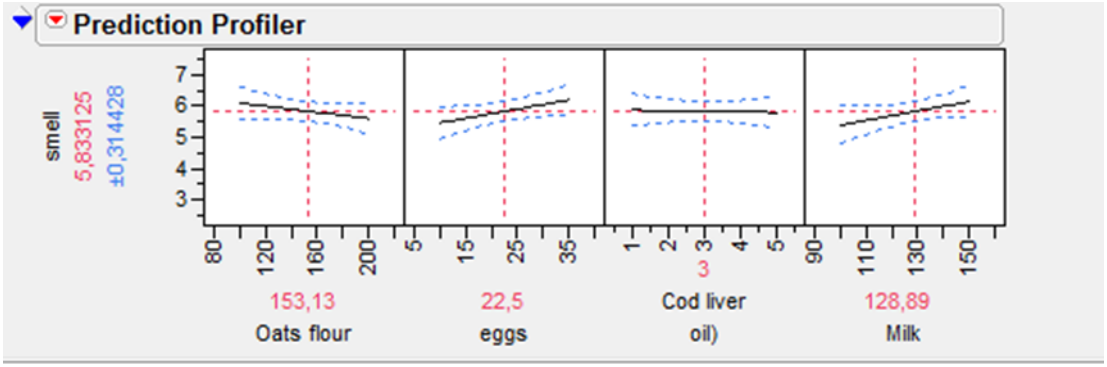

Actual by Predicted Plot
flavor sweet Actual
flavor sweet Predicted
P=0,0069 RSq=0,96 RMSE=0,3788


aroma
5,693382
±0,197635
150
Oats flour
22,5
eggs
3
Cod liver
oil)
128,89
Milk


Actual by Predicted Plot
flavor sweet Actual
flavor sweet Predicted
P=0,0069 RSq=0,96 RMSE=0,3788


Prediction Profiler
flavor sweet
5,18258
±0,249009
153,13
Oats flour
22,5
eggs
3
Cod liver oil)
128,125
Milk


Actual by Predicted Plot
smell Actual
7
6
5
4
3
2,5 3,0 3,5 4,0 4,5 5,0 5,5 6,0 6,5 7,0
smell Predicted P=0,0084
RSq=0,96 RMSE=0,4784


Prediction Profiler
smell
5,833125
±0,314428
7
6
5
4
3
80 120 160 200
5 15 25 35
1 2 3 4 5
3
90 110 130 150
153,13
Oats flour
22,5
eggs
Cod liver
oil)
128,89
Milk

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