"Lucian Blaga" University of Sibiu
Faculty of Agricultural Sciences, Food Industry and Environmental Protection

DOCTORAL DISSERTATION

Summary

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Researches regarding the production of food products with high nutritional value and low saturated fat content by using some vegetal powders

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THESIS STRUCTURE

The thesis entitled " Researches regarding the production of food products with high nutritional value and low saturated fat content by using some vegetal powders" includes the results of the research conducted between 2014-2017 that are presented in 119 pages. This paper consists of 8 chapters, 97 figures, 12 tables, 227 bibliographical references and is structured in two main parts: the documentary study and the experimental part.

The documentary study contains three chapters in which are presented:

- recent researches on the production of low fat saturated foods;
- the importance of using vegetable products and by-products to improve the nutritional value of foods;
- the need to reduce the amount of saturated fatty acids;
- theoretical aspects regarding the importance of bioactive and nutritive components

In the experimental part, which contains 5 chapters, are presented:

- the organization of researches, materials and methods used;
- results and discussions;
- partial and final conclusions;
- personal contributions;
- perspectives for research development.

The thesis also includes annexes, list of abbreviations used and lists of figures and tables.

The experimental part was conducted at the **"Lucian Blaga" University of Sibiu**, in the **laboratories of the Faculty of Agricultural Sciences**, **Food Industry and Environmental Protection**, in **the Laboratory of Atomic Absorption and Inductively Coupled Plasma - Optical Emission Spectrometry** from **SCIENT – Research Center for Instrumental Analysis** in Tâncăbeşti and **Gas-Chromatography Laboratory** of the **National Institute for Research and Development in Chemistry and Petrochemistry - ICECHIM** from Bucharest.

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MOTIVATION AND OBJECTIVES OF THE RESEARCH

In recent years, health is influenced by multiple factors, including pollution and unhealthy lifestyles. If pollution occurs most often without our will, inadequate nutrition and sedentarism are factors related to the education of each individual.

Food products have a particular influence on the human body and, depending on how this thing is perceived, these can play a positive role in maintaining health or can cause various diseases.

In recent years, an increase in the awareness of adverse effects due to high fat consumption has been observed, and people who are aware of their health status have approached a diet low in saturated and trans fats (Akin and Kirmaci, 2015). This, has opened the way for a variety of healthy products, which at the same time have good taste and natural ingredients. Thus, the manufacture of low-fat products by using natural ingredients is motivated.

In order to reduce the incidence of diseases such as: hypertension, obesity and high blood cholesterol (McAfee et al., 2010; Ferguson, 2010; Chavan et al., 2016), it is necessary to reduce the consumption of high saturated fat products, and taking this into account, the main objective of this research has been established: the obtaining of a dairy product and a pastry product with low saturated fat content. From this idea, the other objectives of this research arise:

- ❖ The usage of vegetal powders as fat substitutes.
- ❖ The usage of by-products with nutritional and bioactive potential (watermelon rind and pumpkin seed powder / flour)
- ❖ The determination of the content of biologically active substances from the vegetal powders which are the subject of this research.
- ❖ The determination by using specific analyzes of the nutritive components values of the vegetal powders and of the products obtained by their addition.
- ❖ The comparative analysis between the samples obtained without addition and those with the addition of vegetal powders.

Achieving these goals brings value to the current state of the food industry, namely the marketing of healthy products.

Key words: vegetable powders, saturated fats, minerals, total polyphenols, yogurt, muffins

Chapter 1. Documentary study regarding the obtaining of food products with low saturated fat content and improved nutritional value

One of the greatest challenges facing food research lies in maintaining sustainable food production and, at the same time, in providing quality food with added functionality to prevent lifestyle-related illnesses.

Nowadays, consumers are much more aware of the food they consume than the past generations. This increase in knowledge increases proportionally the demand for more nutritious, qualitative and tasty foods. Consumers are more aware of food issues and monitor and adjust what they consume because they have become more concerned about improving their overall health through daily diets.

Researches have shown a relationship between functional components in food, health and consumer welfare. Disease prevention through the increased consumption of functional foods can cause a substantial reduction in medical costs, which makes functional foods also of interest from a socio-economic point of view.

There is also an increase in the awareness of adverse effects due to high fat consumption. Consequently, people aware of their health status have approached a diet low in saturated fats (Akin and Kirmaci, 2015; Tufeanu and Tiţa, 2016).

Chapter 2. Presentation of the vegetal powders used to reduce the saturated fat content and to improve the nutritional value and bioactive principles of yogurts and muffins

2.1. Watermelon rind powder

Recently, the use of fruit and vegetable by-products has increased to reduce environmental pollution. Agricultural and industrial residues are attractive sources of natural antioxidants and dietary fiber (Larrosa et al., 2002).

Watermelon, *Citrullus lanatus*, belongs to the cucumber family (*Cucurbitaceae*) is large, oval, round or elongated (Koocheki et al., 2007). It is rich in vitamins and serves as a good source of phytochemicals (Perkins-Veazie and Collins, 2004).

The therapeutic effect of the watermelon has been reported by many researchers and has been attributed to the antioxidant compounds (Leong and Shui, 2002; Lewinsohn et al., 2005). Citrulline from watermelon rind has antioxidant effects that protect against free radicals. In addition, citrulline is converted to arginine, an essential amino acid for the heart, circulatory system and immune system (Al-Sayed and Ahmed, 2013).

2.2. Pumpkin seeds powder

Pumpkin seeds possess valuable nutritional and medicinal qualities, besides being a source of quality edible oils.

A press cake is the by-product of the mechanical process for extracting pumpkin seed oil. Pumpkin seed cakes are chemically and nutritionally valuable due to the high amount of crude protein, fatty acids, minerals and energy. It is a very cheap byproduct. The use of this by-product is reduced, usually as animal feed. Pumpkin seed cake is an alternative to the manufacture of low-fat products in terms of profitability as well as to the increase in protein content.

2.3. Chia seeds powder

Salvia hispanica L., also known as chia, is a herbaceous plant native from northern Mexico to Guatemala. Its seeds have been widely used by the Aztecs for food and medicine (Ali et al., 2012).

In Mexico, chia seeds are currently used for nutritional and medicinal properties, such as athletes' resistance, appetite suppression, weight loss agents, glycemic control and intestinal regulation (Martínez-Cruz and Paredes-López, 2014). Potential use of the chia seeds as a good source of protein with remarkable thermal stability has been reported (Sandoval-Oliveros and Paredes-López, 2013). Chia seeds contain a significant amount of lipids (approximately 40% of the total weight), almost 60% of lipids containing ω-3 fatty acids. Dietary fibers constitute more than 30% of the total seed weight and about 19% of the seeds contain proteins of high biological value (Ixtaina et al., 2011). Unsaturated ω-3 fatty acids are nutritionally important for health and are beneficial to people suffering from heart disease, diabetes and immune response disorders (McClements et al., 2007).

2.4. *Psyllium* husks

Psyllium, known as a medicinal active natural polysaccharide, is a good source of soluble fiber (70%) and insoluble (17%) (Verma and Mogra, 2015). Its content of soluble fiber is about eight times higher than that of oat bran. Dietary fibers extracted from the plant possesses pharmaceutical properties and can be used in the production of low-calorie foods (Theuissen, 2008).

Researches indicate that the husks are safe enough to be used in functional and nutraceutical foods. FDA (The Food and Drug Administration) approved the consumption of foods containing *Psyllium* due to their health benefits (Leeds, 2009).

Psyllium has been used as a therapeutic agent for the treatment of constipation, diarrhea, irritable bowel syndrome, inflammatory bowel disease, ulcerative colitis, colon cancer, diabetes and hypercholesterolemia. *Psyllium* husks are considered to be a mild and natural laxative that facilitates digestion. It is assumed that dietary fiber in these husks may help in weight management.

Chapter 3. The importance of bioactive and nutritive components from foods

The world continues to face the increasing number of food-related illnesses and of the population lifestyle. This has resulted in increased interest and demand for fortified foods. Phytochemicals, as natural sources of health promoting ingredients, were and are still widely studied by scientists. Although these future foods with bioactive ingredients can improve and maintain a nutritional balance, the use of phytochemicals, nutraceuticals and functional foods requires profound knowledge and in-depth understanding of the complex physicochemical processes occurring in foods and of the effective food manufacturing strategies which can increase the bioavailability of the valuable bioactive ingredients.

Scientists, food and pharmaceutical industries must aproach not only the quality and stability of functional foods, but also to improve consumer education about the effectiveness and safety of the dietary supplements and functional foods that claim health promotion. These are still existing problems that food manufacturers need to face it.

Chapter 4. Materials used to produce food products with low saturated fat content and with improved nutritional value

4.1. Materials used for the production of stirred yogurts

For the manufacture of yogurt the following ingredients were used:

• Cow's milk with 1,5% and 0,1% fat content purchased from the Romanian commercial system.

• Lactic cultures. For yogurt production a YC-X11 cultures mix from CHR.HANSEN were used.

• Fat substitutes: Chia seed powder, pumpkin seed powder and *Psylium* husks powder have been purchased from the market, and watermelon rind powder was obtained in the laboratory by two procedures: lyophilization and dehydration in the oven.

4.2. Muffins. The role of the ingredients and the manufacturing process

In order to manufacture the muffins was used a classic recipe in which flour was replaced by 10% with maize starch in order to reduce gluten and stabilize air bubbles formed during baking. In order to obtain the dough with specific characteristics, it was intended to limit the formation of the gluten network. For this purpose, the flour has been added to the final stages of the dough preparation.

The fat substitutes used to reduce the amount of butter by 50% and 100% of the muffin samples were in the form of gel (Psyllium gel, chia gel) and paste (pumpkin paste, paste from shell of watermelon). Preparation of fat substitutes consisted of hydration of the powders in milk (0.1% fat).

Chapter 5. Analysis of the vegetal powders to determine the nutritional properties and bioactive principles that they possess

In this chapter, vegetal powders were analysed to determine the micro and macro content, as well as to determine total polyphenolic content and antioxidant activity.

The methods by which the mineral salts were determined were Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) for Ca, K, Na, Mg, Mn, P, Zn, Cu, Fe and Atomic Absorption Spectrometry, FIAS furnace technique for Se.

In the ICP-OES technique, identification of the sample element is done through the radiation wavelength, and the element's concentration is proportional to the radiation intensity, which is recalculated internally from the calibration curve stored in the memory. The FIAS furnace technique uses a combination of the flow injection technique with the detection and atomization of the graphite furnace.

To determine the total polyphenolic content of plant the vegetal powders, 5 extraction methods, adapted according to the methods used for the extraction of phenolic compounds from plant products, were used. These have been applied in order to establish an efficient extraction process.

The extractions varied according to the extraction time and temperature and solvent ratio. For extraction 1, the separation was performed on the ultrasonic water bath for 30 minutes at 40°C and the solvent ratio methanol: water: 0.12 M hydrochloric acid was 70: 29:1 (v / v / v). Extractions 2 and 3 consisted in higher extraction times, namely 90 minutes at 40°C and 24 hours plus 30 minutes on the ultrasonic water bath at 25°C. Extraction 4 consisted of ultrasound separation for 30 minutes at 80°C. For the extraction 5 a solvent ratio of methanol: water: hydrochloric acid 0.12 M -50: 49: 1 (v / v / v) was used, the other parameters were identical to those presented for the extraction 1.

The total polyphenolic content was performed according to the Folin-Ciocalteu method adapted after the European Pharmacopoeia, edition 8.0.

Determination of antioxidant activity was achieved by the DPPH radical scavenging method. The method used to determine antioxidant activity was adapted according to the method applied by Tylkowski et al. (2011) for ethanolic extracts of *Sideritis ssp. L.*

Results and discussions

The total content of polyphenols from the vegetal powders

From figure 1 it can be observed that chia seeds powder has the highest amount of total polyphenols, followed by the watermelon rind powder and the pumpkin seed powder. Reduced amounts of polyphenols are found in the Psyllium husks powder.

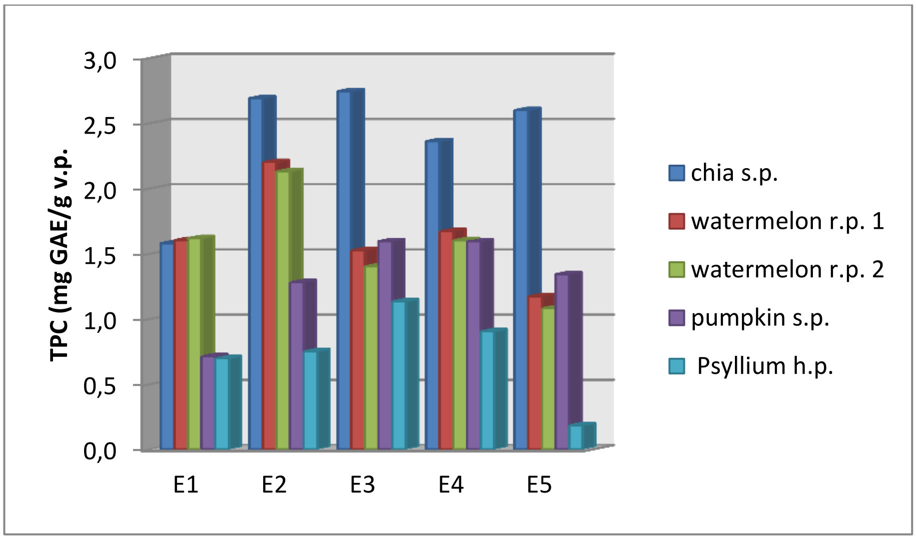

Fig. 1. *The total polyphenols content of the vegetal powders*

Total polyphenolic content values obtained for chia seed by extractions 2, 3, 4 and 5 are higher compared to the data presented by Saphier et al. (2017): 1.99 mg GAE / g v.p. and those obtained by Yi Ding et al. (2017): 2.39 mg GAE / g v.p. and Martínez-Cruz and Paredes-López (2014): 1.64 mg GAE / g v.p.

For watermelon rind powders obtained by oven drying (watermelon r.p. 1) and by lyophilization (watermelon r.p. 2) the maximum values for the total polyphenolic content were determined through the extraction 2.

The pumpkin seed powder has the highest total polyphenolic content for extractions 3 and 4, respectively 1,59 mg GAE / g v.p. The total content of polyphenols determined in the present study is higher compared to the values reported in the literature: 0 , 82 mg GAE / g v.p. and 1.13 mg GAE / g v.p. for flour from two pumpkin species, the Junona and Miranda varieties, both belonging to the C. pepo species (Nawirska-Olszanska et al., 2013) and 0.72 mg GAE / g v.p. (Kiat et al., 2014).

For *Psyllium* husks powder the highest concentration of total polyphenols was determined for the extraction of 3 - 1.13 mg GAE / g v.p., the method which involved the longest extraction period. For extraction 5, a value of 0.18 mg GAE / g v.p. was recorded, which may be mainly due to the ratio of solvents used in MeOH: H_2O: HCl - 50: 49: 1 and the fact that the husks have a high water absorption capacity.

The mineral content of vegetal powders

From the data presented in Table 1, it can be observed that the chia seeds powder contains significant amounts of phosphorus, potassium, calcium and selenium. Concentrations in these minerals are higher than the levels determined by Pereira Da Silva (2016) in chia seeds powder grown in different areas of Brazil.

Pumpkin seeds powder contains the largest amounts of Mg, Zn, Cu, Mn and P in comparison with the other powders analysed. By comparing the values presented by Karanja et al. (2013) we can observe that for Ca, K, Zn and Mg the content is higher in the present research. The most abundant mineral in the Psyllium husks powder is potassium. In the watermelon rind powder, potassium, calcium, phosphorus and magnesium were found in the largest amount.

Table 1. *Minerals concentration of various vegetal powders*

Minerals (mg/100 g)	SAMPLES				
	chia s.p.	**pumpkin s.p.**	**Psyllium s.p.**	**Watermelon r.p. 1**	**Watermelon r.p. 2**
Ca	703,21	151,59	300,38	353,34	372,00
Cu	1,60	1,89	0,27	0,43	0,28
Fe	6,70	14,57	27,71	2,66	3,07
K	794,30	1624,30	1062,17	2998,48	3065,63
Mg	380,49	1034,95	55,68	181,56	234,94
Mn	4,31	8,05	1,41	1,02	1,42
Na	2,38	1,91	88,88	21,34	26,17
Zn	4,55	11,05	0,78	1,97	1,34
P	999,12	2268,62	70,32	317,19	328,44
Se	0,0579	0,0249	0,0147	0,0030	0,0041

* **Watermelon r.p. 1**- powder from watermelon rind, dehydrated at oven
Watermelon r.p. 2- powder from watermelon rind lyophilised

According to the nutritional values provided in Regulation 1169 from 2011, the percentages of minerals for vegetal powders from the reference consumption (RC), which are presented in Figure 2, were calculated. Chia seed powder can provide significant amounts of Ca (87.90%), Mg (101.46%), Se (105.26%) as well as P, Cu and Mn (142.73%, 159.80% and

215.49%). The Psyllium husks powder can provide important amounts of K (53.11%), Mn (70.59%) and Fe (197.94%).

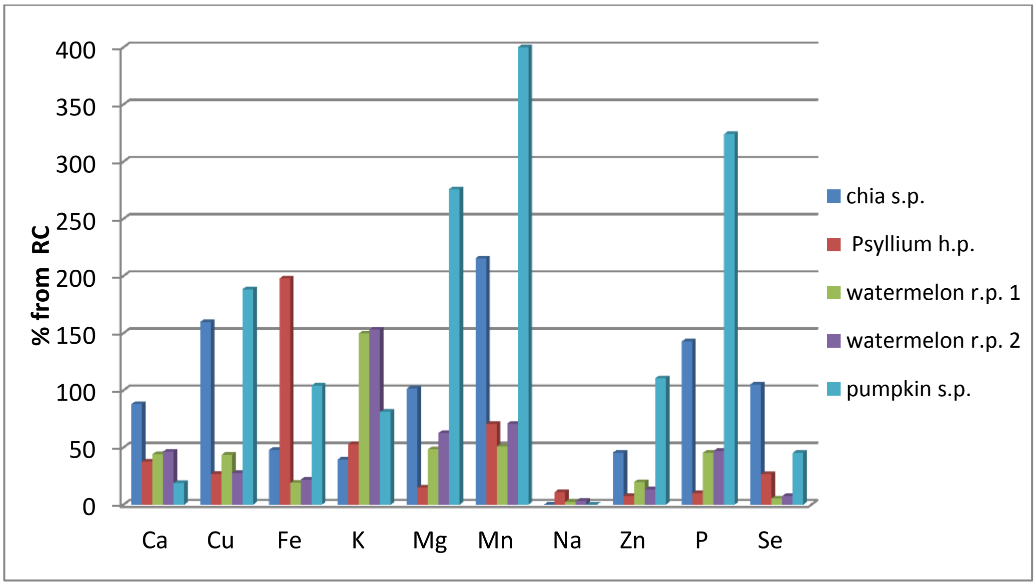

Fig. 2. *The mineral content of the vegetal powders from the reference consumption*

With regard to the quantities of minerals from the reference consumption for watermelon rind powders, it can be observed that about 50% of the Ca, Mg, Cu, Mn, and P requirements can be ensured. The Fe and Zn quantities can be approximately 20% of the RC.

Compared to the other powders, pumpkin seeds can provide the highest amounts of Mg (275.99%), Cu (188.51%), Zn (110.54%), Mn (402.41%) and P (324.09%). Also, the amounts of Fe, K and Se are significant, namely 104.05%, 81.21% and 45.34%.

The antioxidant activity of the vegetal powders

Chia seeds powder has the highest antioxidant activity - 38.77%. This powder is followed by the watermelon rind powder with antioxidant activity of ≈ 15% (Figure 3). The antioxidant activity determined for the pumpkin seed powder is 12.94%. The value of the antioxidant activity obtained for the Psyllium husks powder is 12%.

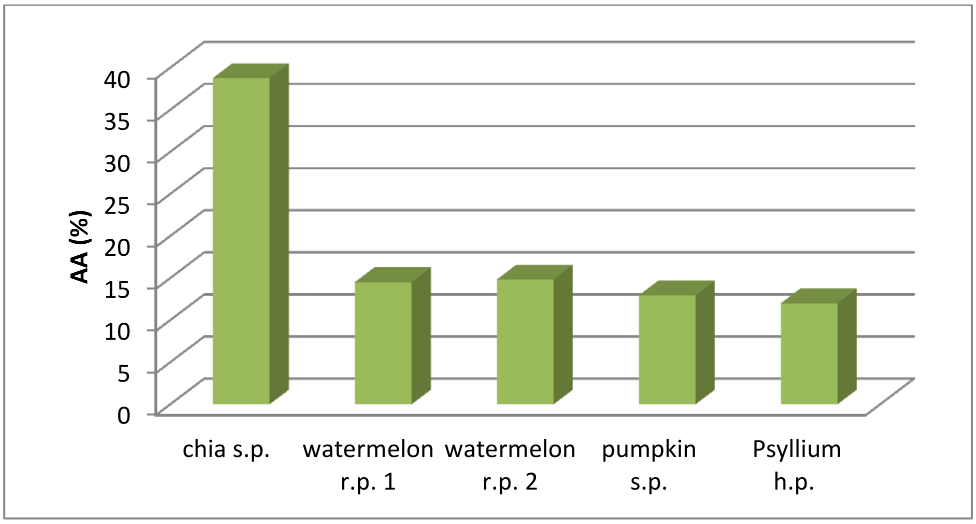

Fig. 3. *The antioxidant activity of the vegetal powders*

Partial conclusions

The chia seeds powder showed the highest total polyphenolic content. This is followed by the watermelon rind powder.

Regarding the cost-effectiveness of methods of obtaining watermelon rind powder, we could say that, due to reduced costs, oven drying can be an effective method to exploit the bioactive and nutritive potential of this by-product.

Among the extraction processes used, the most efficient were extraction 2 and 3.

With regard to the mineral salts content, the powders analysed show considerable amounts of K, P, Mg and Se. Pumpkin seed powder contains the largest amounts of Mg, Zn, Cu, Mn and P in comparison with the other powders analysed. The minerals found in the largest amount in the watermelon rind powder are K, Ca, P and Mg.

The values obtained for the antioxidant activity indicated the chia seeds powder with the highest antioxidant content.

Chapter 6. The study of the main characteristics of the stirred yogurts

In this chapter are presented the results of the analyses performed in order to determine the main characteristics of the stirred yogurts and added vegetal powders.

- Determination of titratable acidity was performed according to ISO / TS 11869: 2012 (Fermented milks - Determination of titratable acidity - Potentiometric method)
- STAS 8201-82 (Milk and Dairy Products - Determination of pH value) has been consulted for pH determination.
- For the determination of the dry matter content, the oven-drying method is used according to SR ISO 6731/96.
- Rheological determinations were performed at 4 ± 2 ° C using HAAKE 550 viscometer, VT model, with concentric cylinder with MV DIN sensor.
- Analysis of the physicochemical and rheological characteristics allowed the selection of a required amount of powder to obtain a low fat fermented dairy product.

The selected products were analysed for the determination of the mineral salts composition as well as for the determination of the total content of polyphenols and the antioxidant activity.

Results and discussions

Titratable acidity variation

The highest acidity values were observed in samples with added pumpkin seed powder and watermelon rind powder, the maximum values ranging between 122 ° T-139 ° T, depending on the amount of added powder (0.5 -1.5%).

Increased acidity has been reported previously by the addition of other hydrocolloids or polysaccharides such as guar gum in low fat frozen yogurt desserts (Milani and Koocheki, 2011; Sekhavatizadeh and Sadeghzadehfar, 2013) and native and modified starches (Lobato-Calleros et al., 2014).

Dry matter content variation

During the 14 days of storage, all yogurt samples show a slight increase in the dry matter content.

Dry substance is an important parameter in the quality of yogurt. A correlation can be made between the dry matter content and other two important yogurt properties: the degree of syneresis and viscosity.

The increase in dry matter in fermented dairy products increases the firmness and viscosity of yogurt and decreases syneresis (Hanif et al., 2012).

The mineral content of yogurt samples with added vegetal powders

Yogurt samples can provide important amounts of calcium and phosphorus (Figure 4). For example, the consumption of 100 g of yogurt can provide about 14% Ca of the recommended daily requirement. Regarding the amount of phosphorus with which yogurt can contribute to ensuring the daily dose, we can observe that with 0.5% added pumpkin seed powder comes with the highest contribution, namely 15.99%. The yogurt with *Psyllium* powder can provide 14.15% P, and the other samples can contribute with about 15% P.

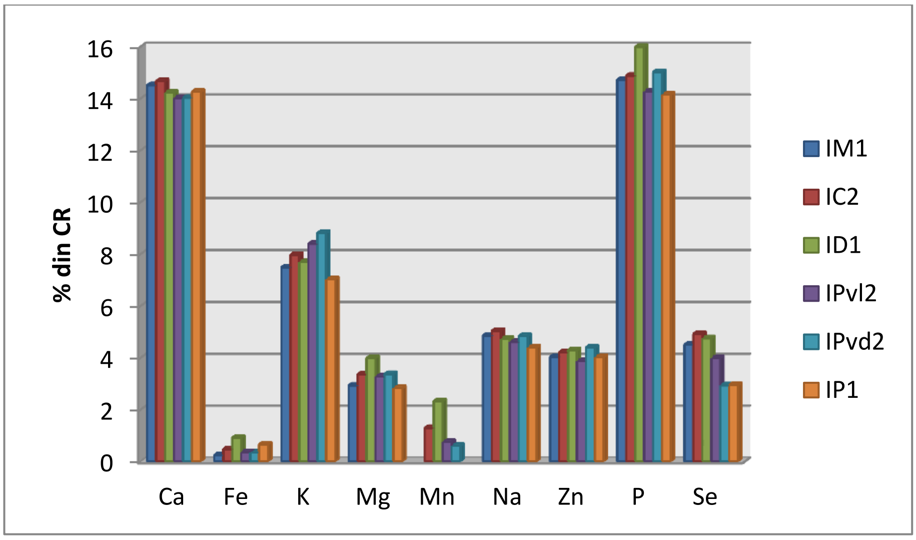

Fig. 4. *The content of minerals of the yogurt samples from the reference consumption*

The total phenolic content of yogurt samples with added vegetal powders

The yogurt obtained with 0.5% chia seed powder has showed the highest content of polyphenols (3.05 mg GAE / 100 g p.). The yogurts obtained with lyophilized and dehydrated at oven watermelon rind powder have the following values for the total polyphenols content: 2.57 mg GAE / 100 g p. and 2.76 mg GAE / 100 g p.

The total content of polyphenols for the yogurt with 0.5% pumpkin seed powder was 1.74 mg GAE / 100 g p. The lowest values of the total polyphenolic content were recorded by the yogurt with 0.3% Psyllium husks powder - 0.23 mg GAE / 100 g p.

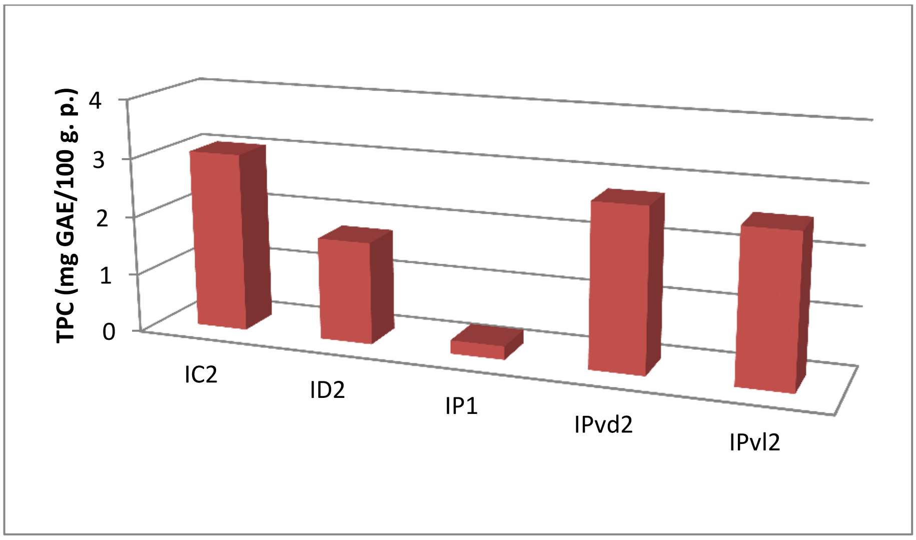

Fig. 5. *The total phenolic content of yogurt samples with added vegetal powders*

The antioxidant activity of the yogurt samples with added vegetal powders

The yogurt with 0.5% chia seed powder recorded the highest antioxidant activity - 8.85%, followed by yogurt with 1% added lyophilized and dried at oven watermelon rind powder with AA values of 8, 22% and respectively 8.54%.

The antioxidant activity of the yogurt with pumpkin powder was 6.02%, and of the yogurt with 0.3% Psyllium husks - 4.76%.

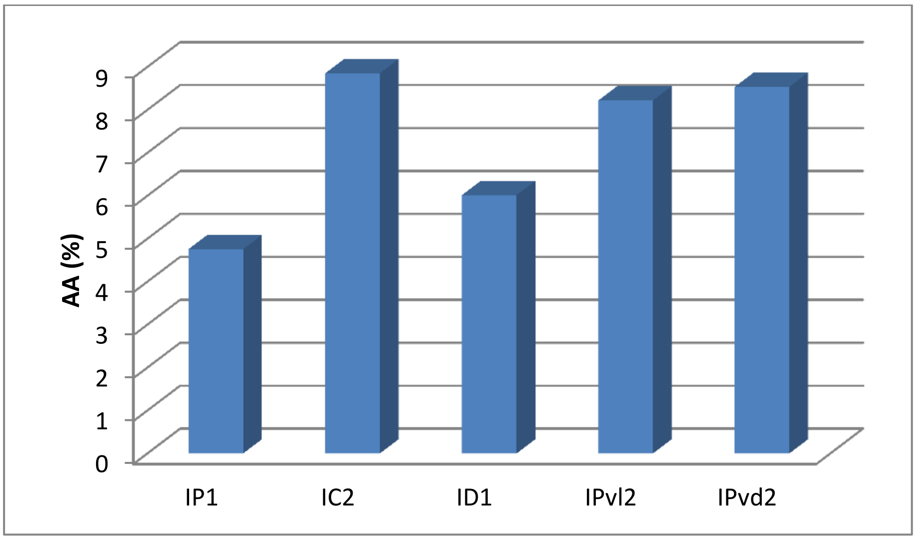

Fig. 6. *The antioxidant activity of the yogurts with added vegetal powders*

To these values of antioxidant activity have contributed factors, such as: the amount of added powder that comes with an antioxidant intake, including: polyphenols, certain vitamins; the amount of vitamins with antioxidant properties found in raw milk and the method of extraction.

Partial conclusions

During storage, for all the yogurt samples it has been found that the increase in titratable acidity occurs in correlation with the lowering of the pH.

During the 14 days of storage, all yogurt samples show a slight increase in the dry matter content.

After studying the rheological properties it was found that for all yogurt samples there was an increase in viscosity during the storage period. This is due to the restructuring of the yogurt gel. It has also been observed that with the increase in shear rate, the viscosity decreases. This behaviour is typical for non-Newtonian fluids and is clearly evidenced for yogurt samples obtained by the addition of vegetal powders.

The study of the viscosity samples also allowed the selection of the required amount of powder to be used as a fat substitute to obtain a fermented dairy product with viscosity values close to those of the sample with 1.5% fat. All these findings on the variance of viscosity versus the amount of added powder together with the results of the physicochemical determinations contributed to the determination of the following quantities of vegetal powders as necessary to obtain a product with the desired characteristics: 0.5% chia seeds powder, 0.5% pumpkin seeds powder, 0.3% *Psyllium* husks powder and 1% watermelon rind powder.

Among the benefits of using these substitutes are the reduction in saturated fat content and the improvement in the content of mineral salts and polyphenols, important compounds for maintain the health at normal parameters.

Chapter 7. The study of the main characteristics of the muffin samples

In this chapter are presented the results of the analyses conducted to determine the main characteristics of the muffins produced by using fat substitutes based on vegetal powders..

- Determination of the humidity of the muffin samples was performed according to SR 91/2007.
- The volume determination was performed by the gravimetric method according to SR 91: 2007.
- Determination of water activity. The water activity represents the free water from a product, because a percentage of it can be bound to proteins, carbohydrates, etc., and thus the water bounded is not available to the growth of microorganisms. The water activity of the foods affects the microorganism's multiplication.
- For the sensory evaluation of the muffin samples, 17 untrained panelists, between the ages of 15 and 59 were asked to score the samples in terms of appearance, color, porosity, taste and sweetness. A hedonic scale of 7 points was used to evaluate the sensory attributes of muffins, where 1 = very unpleasant and 7 = very pleasant.
- Determination of the fatty acid composition of the muffin samples was performed according to the method optimized by Galan et al. (2017). The fatty acid methyl esters were analyzed by a GC-MS 7000 TRIPLE QUAD system (Agilent Techologies, USA).

Results and discussions

The moisture variation of muffin samples during storage

It can be observed that through the use of fat substitutes has increased the moisture content of the samples. The highest humidity values were recorded for the samples where the butter was completely replaced (Figure 7).

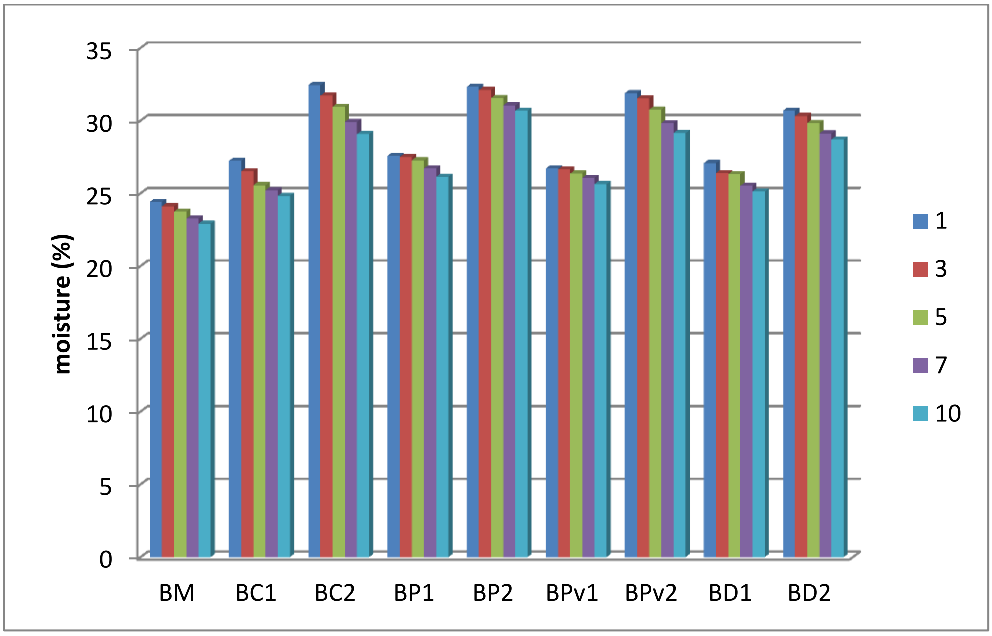

Fig. 7. *The humidity of the muffin samples*

Similar humidity values obtained in the present study have been reported by Jauharah et al. (2014) for muffins obtained by replacing the wheat flour with baby corn powder. The samples with wheat flour had an initial moisture content of 25.23%, but by replacing it with baby corn powder, the moisture increased, so for muffins with 30% flour replacement, a moisture content of 30.44% was obtained.

The replacement of butter has led to the production of high moisture muffins, which can be attributed to the large quantities of water derived from the incorporated vegetal pastes and gels and to the high water affinity of the substitutes used.

The volume variation

Compared to the control sample, the volume of muffin samples increased with the increase in the amount of fat replacement. The formation of the larger air cells during baking results in a higher height and volume.

A number of interdependent factors influence the volume: the rheological properties of the dough (affected by the ingredients), the degree of air incorporation, the time and the speed of mixing the ingredients (Martínez-Cervera et al., 2011).

The variation of water activity for muffin samples during storage

For all the muffin formulations, water activity was higher compared to control. Wekwete and Navder (2007) also noticed that the water activity was greater for biscuits prepared with avocado puree compared to the blank sample.

For all samples, the increase aw is noted with the increase of the storage period. Similar results were reported by Bhise and Kaur (2015) for muffins made with fibres from oat, *Psyllium* and barley.

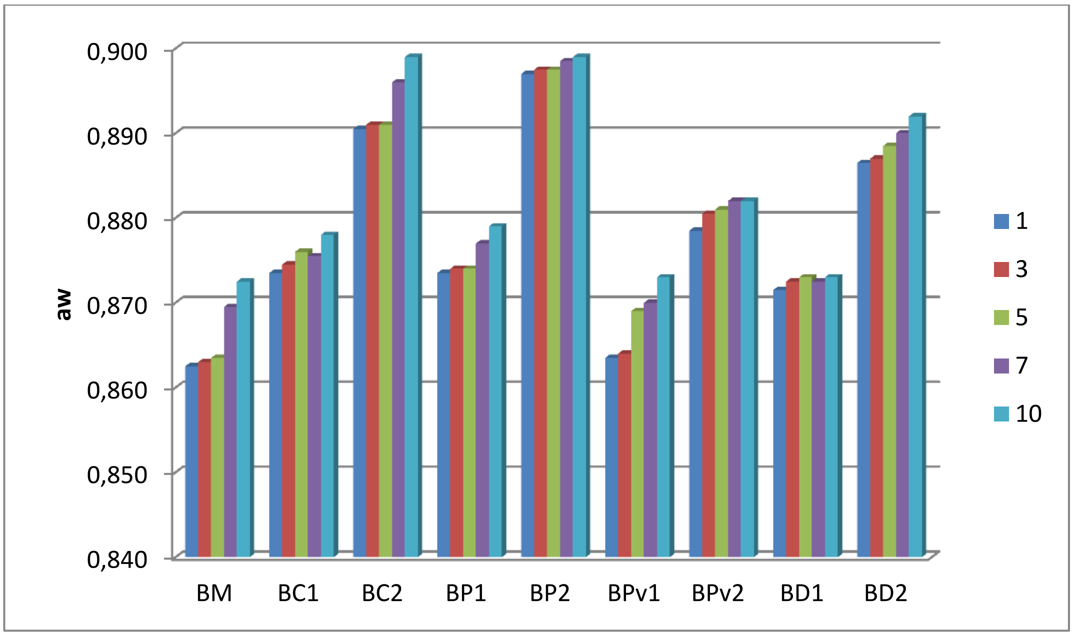

Fig. 8. *Water activity for muffin samples*

The muffin samples showed values of water activity greater than 0.7 – the limit for the moulds development (Andrade et al., 2016). This indicates for all samples a critical stability during storage for 10 days, because they have values of aw greater than 0.863, which may favour the development of gram positive and negative bacteria, yeasts and moulds.

In order to avoid the development of microorganisms, the quality of the raw materials and the hygienic conditions during processing and in particular after obtaining the product must be taken into account.

The mineral content of muffin samples

In the case of muffins with chia seeds powder, a significant increase in the amounts of P, Ca, Zn, K and Mg compared to the control sample (Table 2) is noted. This is due to the important mineral content that the chia powder possesses.

Table 2. *The mineral content of muffin samples*

Minerals (mg/100 g)	**Samples**								
	BM	**BP1**	**BP2**	**BC1**	**BC2**	**BPv1**	**BPv2**	**BD1**	**BD2**
Ca	65,570	83,380	101,000	88,170	113,600	81,980	100,000	81,300	95,750
Cu	0,147	0,105	0,101	0,117	0,125	0,101	0,094	0,138	0,169
Fe	1,193	1,223	1,443	1,219	1,364	1,157	1,204	1,495	1,759
K	151,200	162,000	179,900	165,200	196,600	196,700	262,300	179,600	226,300
Mg	17,880	20,150	22,660	23,910	29,480	23,320	26,610	44,380	70,020
Mn	0,287	0,282	0,312	0,327	0,370	0,301	0,312	0,474	0,656
Na	556,200	569,800	630,200	586,400	620,800	556,900	557,400	571,100	586,200
Zn	0,776	0,817	0,886	0,897	0,971	0,880	0,891	1,077	1,375
P	340,300	353,000	384,800	377,900	409,600	345,000	367,300	396,900	457,300
Se	0,009	0,010	0,011	0,010	0,012	0,011	0,012	0,010	0,012

It can be noticed that the muffins with pumpkin flour paste and chia seeds gel present the highest P values, due to the contribution of the added vegetal powders.

Muffins produced by using the watermelon rind powder have the highest K content.

For the muffins obtained with pumpkin seeds powder, is observed an increase in magnesium, zinc, phosphorus, potassium and zinc content.

The samples with *Psyllium* husks gel also presented an improved nutritional value compared to the control sample. This addition comes with an important contribution to the magnesium and phosphorus content.

Sensory analysis of muffin samples

The smallest values for sweetness were obtained in the case of muffins with watermelon rind paste.

The appearance of the muffins was one of the attributes that did not vary significantly with the use of fat substitutes.

The porosity of the analysed products was affected by the reduction in butter content. This characteristic is mainly influenced by the quality and quantity of gluten from flour and its ability to form and retain the gases and at the same time by the presence of fats to disrupt the gluten network, in order to produce final products with a uniform structure and high volume.

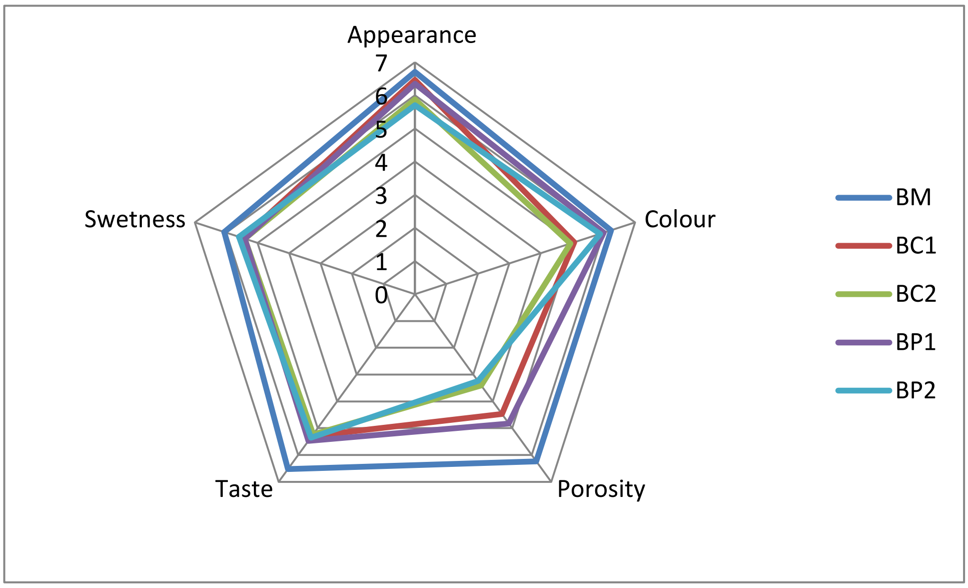

Fig. 9. *The results of the sensorial analysis of the muffin samples with gel from chia seeds and Psyllium husks*

Another sensory characteristic influenced by the use of vegetable powders as fat substitutes was the colour. In the case of samples made with watermelon rind powder, the lowest values for this characteristic were recorded.

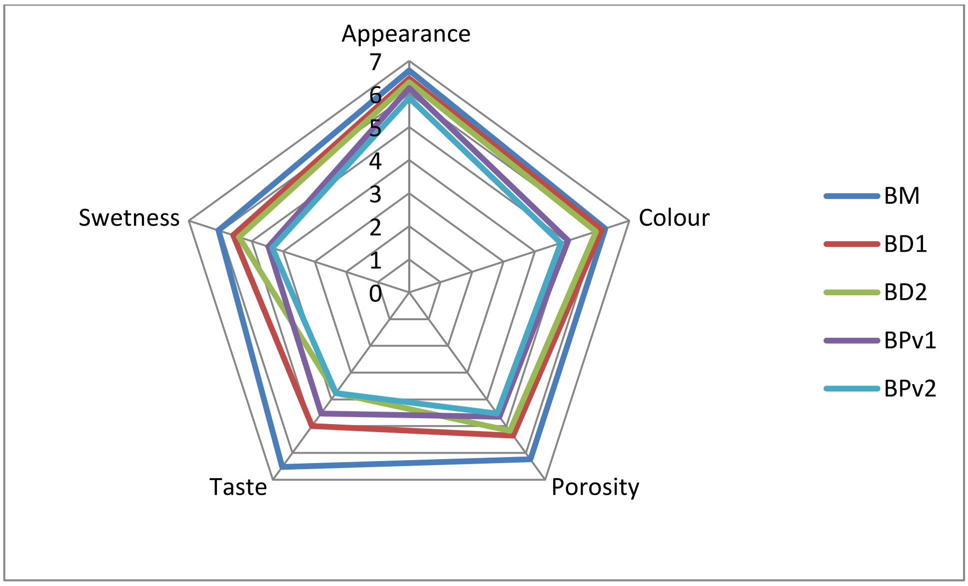

Fig. 10. *The results of the sensorial analysis of the muffin samples with powder from watermelon rind and pumpkin seeds flour*

For samples with chia and Psyllium gel muffins, the taste was moderately pleasant and pleasant, indicating that the powders used did not impart a bad or disturbing taste to the products (Figure 9). In comparison, for the samples in which the butter was totally replaced by the use of pumpkin flour and watermelon rind powder, the taste was perceived to be slightly unpleasant.

Based on the sensory analysis it can be said that the reduction of the fat content by 50% obtained results with a greater degree of acceptability for taste, appearance, porosity and colour compared to the total replacement of fat.

Total phenolic content of muffin samples

From Figure 11 it can be observed that the use of fat substitutes has resulted in an increase in the content of total polyphenols. Muffin samples with chia gel have the highest content of polyphenols - 77.43 mg GAE / 100 g d.m., followed by the muffins with watermelon rind paste - 72.07 mg GAE / 100 g d.m. For the products obtained by adding pumpkin flour, a total polyphenolic content of 63.63 mg GAE / 100 g d.m. was determined. The muffins with a butter reduction of 50% by using Psyllium husks gel have a total polyphenolic content of 61.33 mg GAE / 100 g d.m.

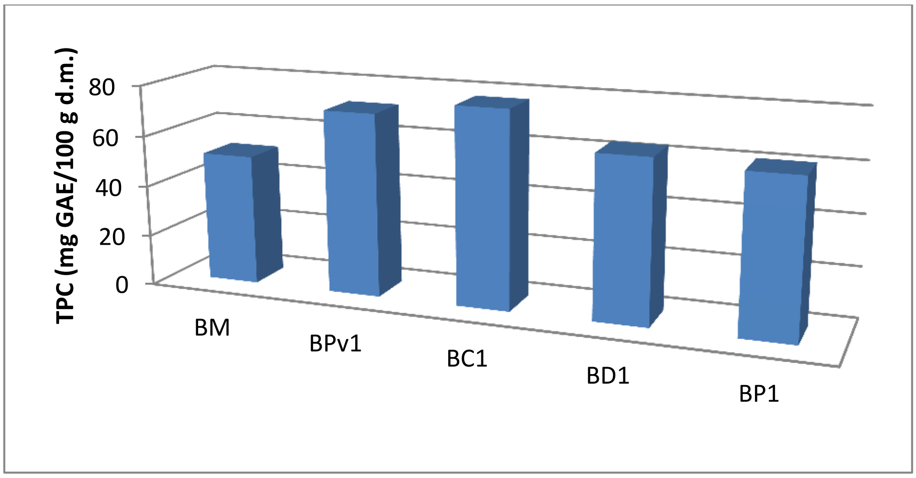

Fig. 11. *The content of total polyphenols of the muffin samples*

The total fat content of the muffin samples

According to figure 12 it can be observed that the control sample contains the highest fat content (≈ 14%) compared to the other samples where the butter was reduced by 50% by the use of the fat substitutes based on vegetal powders. the muffins with chia seeds gel have a fat content of 9.14%, with 32.89% lower than the control sample. By replacing 50% butter with pumpkin seeds paste was obtained a reduction by 39.65% total fat. In the case of muffins with Psyllium gel, the lowest value of the total fat content was obtained (7.7%).

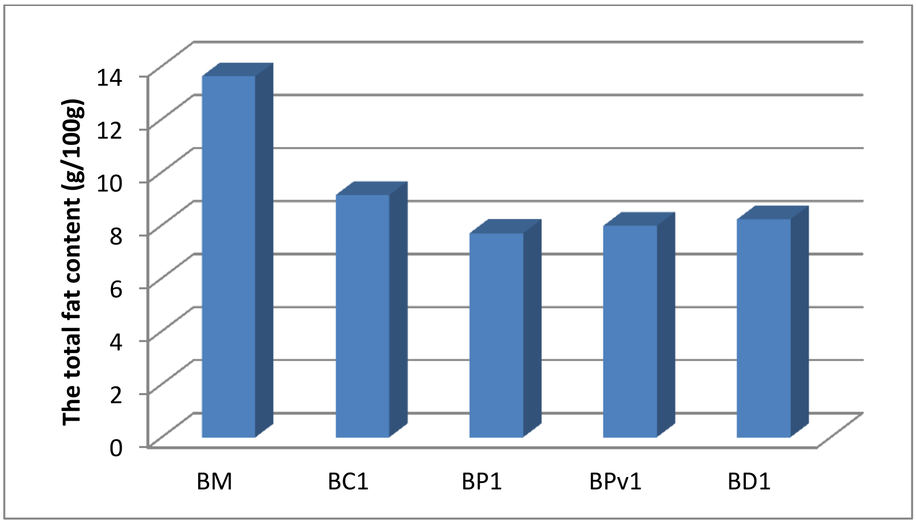

Fig. 12. *The total fat content of muffins*

With regard to the total fat content of muffins obtained by 50% reduction of butter with dehydrated watermelon rind, a total fat content of 7.98% is noted.

By replacing the butter with Psyllium gel, the most significant reduction in total fat (43.47%) was achieved. The second significant reduction in total fat was obtained by using the watermelon rind paste (41.40%).

The fatty acids content of the muffin samples

The fatty acid identified in the highest amount is palmitic acid (C16: 0). It is considered to be the most abundant saturated fatty acid.

In the muffin samples in which the watermelon rind paste was added to replace the butter, the reduction in saturated fatty acids was achieved by 46.30%.

For the muffins samples in which the *Psyllium* husks gel was used, there was a decrease in the saturated fat content by 47.69%. By using this fat substitute, the greatest reduction in the amount of saturated fatty acids was achieved. This is mainly due to the composition of the bran, which does not contribute with fat to the finished product. The amount of saturated fatty acids in muffins with pumpkin seeds paste was reduced by 41.22%.

In the case of muffins with chia seeds gel, the amount of saturated fatty acids was reduced by 36.49%. By using this fat substitute, the smallest reduction in the amount of saturated fatty acids was achieved. This is mainly due to the composition of chia seed, known for its high fat content.

Table 3. *The fatty acids content of the muffin samples*

	Name of fatty acids	Retention time	Fattyy acids (g/100 g p.)				
			BM	**BPv1**	**BP1**	**BD1**	**BC1**
Saturated fatty acids	**capric acid**	6,858	0,16	0,13	0,07	0,16	0,18
	lauric acid	10,155	0,48	0,24	0,22	0,29	0,33
	miristic acid	13,405	1,53	0,73	0,74	0,83	0,94
	pentadeciclic acid	14,915	0,18	0,08	0,08	0,09	0,11
	palmitic acid	16,445	4,71	2,55	2,52	2,79	2,96
	stearic acid	19,16	1,61	0,91	0,90	0,93	0,99
	Total		**8,66**	**4,65**	**4,53**	**5,09**	**5,50**
Unsaturated fatty acids	**miristoleic acid**	13,188	0,14	0,06	0,06	0,08	0,09
	palmitoleic acid / omega 7	16,079	0,30	0,16	0,16	0,20	0,21
	linoleic acid	18,713	0,43	0,58	0,26	0,35	0,29
	elaidic acid	18,849	3,73	2,42	2,49	2,29	2,66
	conjugated linoleic acid (9,11)	19,397	0,22	0,07	0,11	0,12	0,25
	conjugated linoleic acid (10,12)	19,505	0,14	0,03	0,08	0,09	0,15
	Total		**4,96**	**3,33**	**3,17**	**3,13**	**3,64**

Partial conclusions

The replacement of butter has led to the production of high moisture muffins, which can be attributed to the large quantities of water derived from the incorporated vegetal pastes and gels and to the high water affinity of the substitutes used.

The muffin samples showed values of water activity greater than 0.7 – the limit for the moulds development. This indicates for all samples a critical stability during storage for 10 days, because they have values of aw greater than 0.863, which may favour the development of gram positive and negative bacteria, yeasts and moulds.

In order to avoid the development of microorganisms, the quality of the raw materials and the hygienic conditions during processing and in particular after obtaining the product must be taken into account.

By using the vegetal powders, a fortification of the products with numerous mineral salts can be achieved.

Based on sensory analysis we can say that vegetal powders used may be promising options for reducing the fat content. The results obtained indicated the muffin samples obtained by reducing the butter by 50% are preferred.

The results of quantitative determination of fatty acids indicated that there was a significant reduction in the saturated fat content, the values being between 36.49% and 47.69%.

Chapter 8. Final conclusions

The awareness of the negative effects that a diet rich in saturated fatty acids has on health, the need to produce functional products for improving and maintaining health and the importance of using secondary products are the main factors that determined the objectives of this research.

The highest total polyphenolic content was determined for chia seeds powder, followed by watermelon rind powder.

Due to reduced costs, oven drying can be an effective method to exploit the bioactive and nutritive potential of this secondary vegetal product.

With regard to the extraction methods used, the most efficient were extractions 2 and 3.

With regard to the mineral salts content, the powders analysed show considerable amounts of K, P, Mg and Se. Pumpkin seed powder contains the largest amounts of Mg, Zn, Cu, Mn and P in comparison with the other powders analysed. The minerals found in the largest amount in the watermelon rind powder are K, Ca, P and Mg.

In the *Psyllium* husks, the most abundant mineral is potassium, and the chia seeds powder contains significant amounts of P, K, Ca and Se.

For all the yogurt samples an increase in titratable acidity in correlation with the pH lowering and also a slight increase in the dry matter content were found.

After studying the rheological properties it was found that for all yogurt samples there was an increase in viscosity during the storage period. This is due to the restructuring of the yogurt gel. It has also been observed that with the increase in shear rate, the viscosity decreases. This behaviour is typical for non-Newtonian fluids and is clearly evidenced for yogurt samples obtained by the addition of vegetal powders.

The study of the viscosity samples also allowed the selection of the required amount of powder to be used as a fat substitute to obtain a fermented dairy product with viscosity values close to those of the sample with 1.5% fat. All these findings on the variance of viscosity versus the amount of added powder together with the results of the physicochemical determinations contributed to the determination of the following quantities of vegetal powders as necessary to obtain a product with the desired characteristics: 0.5% chia seeds powder, 0.5% pumpkin seeds powder, 0.3% *Psyllium* husks powder and 1% watermelon rind powder.

To highlight the health benefits of selected yoghurt, in addition to the fat content reducing, the content of mineral salts, the total amount of polyphenols and antioxidant activity were determined. The results indicated that the use of the vegetal powders led to the improvement of these characteristics for all samples. Yogurt obtained with 0.5% chia seeds powder showed the highest values of these parameters analysed, followed by yogurts with lyophilized and dried at the oven watermelon rind.

The muffins produced by reducing butter with fat substitutes based on vegetal powders have been characterized by a high moisture content, which can be attributed to the large quantities of water derived from the incorporated vegetal pastes and gels and to the high water affinity of the substitutes used.

The muffin samples showed values of water activity greater than 0.7 – the limit for the moulds development. This indicates for all samples a critical stability during storage for 10 days.

By using the vegetal powders, a fortification of products with numerous mineral salts can be achieved.

Based on sensory analysis we can say that vegetal powders used may be promising options for reducing the fat content. The results obtained indicated the muffin samples obtained by reducing the butter by 50% are preferred. These samples with appropriate taste, appearance, colour and porosity were selected for the determination of the total polyphenolic content and antioxidant activity, and the results indicated an increase in the value of these parameters. The muffins with chia showed the highest polyphenolic content, followed by the muffins with watermelon rind paste and those obtained with pumpkin flour and *Psyllium* husks gel.

Also, samples of muffins selected after sensory analysis were analysed for the quantitative determination of fatty acids. The results indicated that there was a significant reduction in the saturated fat content of the analysed samples.

The results have indicated that the vegetal powders from chia seeds, pumpkin seeds, *Psyllium* husks and watermelon rind may be favourable options for the production of low-fat stirred yogurt and for replacing the butter in the muffins recipe.

By reducing the amount of saturated fats and by using these substitutes, the risk of some diseases like: high blood pressure, obesity and high cholesterol in the blood can be reduced. Also, due to the antioxidant properties of phenolic compounds, these products can provide protection against diseases such as atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases.

The results provide important insights into the potential use of these vegetal powders for the functional foods production.

8.1. Personal contributions

The personal contributions found in this paper consist in:

- The characterization of vegetal powders in terms of total polyphenols content, antioxidant activity and content of micro and macroelements;
- The use of these powders to produce low fat saturated fat products;
- The utilization of two by-products from indigenous products;
- The identification of an efficient extraction method for the determination of the total content of polyphenols and antioxidant activity.
- The establishing of the required amount of powder to obtain the stirred yogurt with the desired characteristics through a series of physico-chemical and rheological analyses;
- Sensory analysis determination of manufactured pastries to determine the required amount of butter substitute;
- The improving of the nutritional value of products by using the vegetal powders;
- The highlighting the reduction in the saturated fat content of the muffin samples by GC-MS analysis.

8.2. Perspectives for research development

In order to continue the research undertaken in this paper I propose the following actions:

- The production of other functional foods by using vegetal powders;
- The study of the bioactive potential of other plant products through the methods described in this paper.

- The determination of other analysis methods to highlight the nutritional and bioactive potential of the vegetal powders that are the subject of this study, as well as the manufactured foods;
- The analysing of other by-products from fruits and vegetables processing industry and the highlighting the importance of their bioactive and nutritional potential, which is particularly important in maintaining the health status and in reducing the incidence of many diseases;
- The performing of the procedures in order to put the products on the market.

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