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ORIGINAL RESEARCH PAPER

EFFECTS OF SOME VEGETABLE PROTEINS ADDITION ON YOGURT QUALITY

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Abstract: Yogurt is the most famous fermented milk product, with yogurt consumption having grown over the years and is still rising in many countries. The aim of this study was to determine the effects of different vegetable proteins on the rheological, physicochemical and sensorial properties of yogurt with 10 % fat. The studied vegetable proteins were pea protein, soya protein, wheat gluten, hemp protein and pumpkin seed flour. The amount of vegetable protein added to the raw milk before pasteurization was calculated so that the yogurt contained 4 % of the protein. The finished product is a functional food that uses no preservatives, additives or genetically modified organisms. The addition of vegetable proteins in the yogurt formulation modified their rheological, physicochemical and sensory properties. It is possible to produce yogurts containing vegetable proteins without changing the technological procedure and with a good quality for the consumers.

Keywords: *functional product, properties, rheological syneresis, vegetable proteins, water holding capacity*

INTRODUCTION

Worldwide the food and, therefore, dairy market is dynamic and contributes to increased competition due to technical-scientific progress and consumer exigencies. Specialists in the dairy industry attach great importance to diversifying product ranges to meet consumer demand. New product ideas derive from tracking global trends that apply to each region, with manufacturers combining innovation and tradition in developing new products. These products are functional foods with an important role in improving human health [1].

It is known that yogurt contains high levels of protein, a macronutrient with the highest satiating capacity, so it could be an excellent basis for designing a nutritional product [2]. There are three good options to achieve the desired protein and solids contents in yogurt: addition of different proteins, evaporation of water from milk under vacuum or removal of water by membrane filtration [3].

Many researchers have achievements in the field of fortification of protein-based yogurt, with milk proteins or vegetable proteins [4 – 8]. The additions of these proteins lead to the improvement of the quality of yogurt and an increase in its nutritional value. It is known the effect of addition of the whey protein concentrates and Na or Ca caseinates on the textural and physical properties of yogurts and positive effects on yogurt firmness, viscosity and functional properties.

The addition of milk protein could be difficult and expensive. An alternative for improving the yogurt nutritional value is the fortification of yogurt with plant proteins [9]. The firming effect of different proteins addition in the formulation of yogurts could lead to a distinctive structure of the casein, with beneficial influences on the textural characteristics of finished product. On the other hand, for the consumer, the increase in the protein content improves the degree of satiety, especially beneficial for those who consume fermented dairy products for health reasons, certain diets, etc.

The paper proposes the use of 5 vegetable proteins as an alternative to the replacement of milk proteins in the recipe of 10 % fat yogurt. The five vegetable proteins taken in the study are: hemp protein, pumpkin seed flour, pea protein, wheat gluten and soya protein. The first two sources of protein are a novelty in the yogurt recipe, and the use of gluten to obtain yogurt has been very little studied compared to the use of pea protein and soya protein.

Pea is used in the food industry due to its functionality, nutritional properties, availability, a relatively low raw material cost, low allergenicity, and it does not contain genetically modified organism. Pea proteins have been investigated for use in food products, including bakery and dairy products, and texturized protein for meat substitutes and snack foods [10].

Soybeans are unique among crop plants, they supply protein equal in quality to that of animal sources. Soy protein is a primary component in meat analogues consumed by people who prefer foods that are animal-free or lower in saturated fats [11].

Wheat gluten is a co-product of wheat starch in the wheat processing which contains 75 - 85 % proteins and 5 - 10 % lipids of dry matter. Wheat gluten main protein constituents are: gliadin, which contributes primarily to viscous properties and glutenin, which contributes mainly to elastic properties. Gluten is very important in the bakery industry because it forms a continuous three-dimensional network during the dough development process [12, 13]. The use of gluten in the production of fermented dairy products has been very poorly studied [1].

Hemp seeds are traditionally used in food in the form of oil and protein isolates. Hemp protein consists primarily of albumin (33 %) and globulin called edestin (65 %), rich in essential amino acids [14, 15]. Hemp protein contains all nine essential amino acids in a favorable ratio, and easily digested. Hemp is used to obtain a diversity of products from different fields such as agriculture, textile, bio composite, paper-making, automotive, construction, bio-fuel, functional food, oil, cosmetics, personal care and pharmaceutical industry [16].

Pumpkin seeds, as one of oilseeds, are in the attention of specialists because they are a very good source of proteins [17, 18]. Pumpkin seed are antioxidant, anti-inflammation and immunomodulation, anticancer properties, hypolipidemic, antihypertensive, cardioprotective and hypoglycemic effect [19]. The aim of the present work was to relate the rheological, physicochemical and sensorial characteristics of 10 % fat yogurts with different vegetable protein addition.

MATERIALS AND METHODS

Preparation of yogurt samples

Yogurt was made under laboratory conditions from cow's milk with a fat content of 3.5 %, 3 % protein and 4.5 % carbohydrates. For the normalization of yogurt to a fat content of 10 %, fresh cream was used, containing 35 % fat and 2 % protein. All yogurt samples were normalized in terms of protein content 4 % by addition of plant proteins, as follows: hemp protein (50 % protein, 21 % fiber, 11 % lipids from Canah International, Romania); pumpkin seed flour (65 % protein, 10.8 % fiber, 9.3 % lipids from The Hut Group, UK); pea protein isolate (75 % protein, 0 % fiber, 5.0 % lipids from The Hut Group, UK); wheat gluten (84 % protein, 0 % fiber, 1.4 % lipids from Sano Vita, Romania); soy protein isolate (90 % protein, 0 % fiber and 3.3 % lipids from Enzymes & Derivates, Romania). The chemical composition of the ingredients used to obtain the yogurt samples is that indicated by the manufacturer in the technical sheets. All yogurt samples were normalized to the same fat content (10 %) and to the same protein content (4 %), the samples being coded like so:

Table 1. *Manufacturing recipes for yogurt samples with 5 % fat and 4 % protein*

Sample	Code	The formulation of yogurt		
		Milk [%]	Plant protein [%]	Cream [%]
Control sample, yogurt without plant proteins (with milk protein, 1.5 %)	Y_C	79.37	-	20.63
Yogurt with natural hemp protein	Y_{NHP}	76.89	2.79	20.32
Yogurt with pumpkin seed flour	Y_{PSF}	77.65	1.98	20.37
Yogurt with pea protein isolate	Y_{PPI}	77.73	1.72	20.55
Yogurt with wheat gluten	Y_{WG}	77.80	1.48	20.72
Yogurt with soy protein isolate	Y_{SPI}	77.97	1.38	20.65

The control (YC) sample was normalized to the 4 % protein content by the addition of milk protein (85 % protein, 2.5 % lipids from Enzymes & Derivates, Romania).
Prior to inoculation, the ingredients mixture was pasteurized at 85 °C for 30 minutes, and then cooled to 41 °C. For inoculation, it was used the yogurt starter culture DI–PROX, Enzymes & Derivates, composed of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The samples were inoculated with 0.2 % (w/v) yogurt starter culture and incubated at 41 °C until the *p*H reached 4.6. The yogurt samples were stored at 4 °C for 24 h, and then the analyses were performed in order to determine the rheological, physicochemical and sensorial properties.

Physicochemical analysis of yogurt samples

The modifications of yogurt samples' *p*H during fermentation were determined using a portable F2 Standard Mettler Toledo pH-meter (METTLER TOLEDO, Germany). The titratable acidity of the yogurt samples was expressed as % lactic acid according to AOAC (2005) [20].
Syneresis of yogurt samples was determined according to the method of Barkallah *et al.* (2017) by placing 100 mL of each sample in a funnel lined with Whatman filter paper number 1 [21]. After 6 h of drainage, the volume of whey was measured and following formula was used to calculate susceptibility of syneresis:

$$S = (V_1 / V_2) \times 100 \quad (1)$$

where: V_1 = volume of whey collected after drainage; V_2 = volume of yogurt sample.
Water holding capacity of yogurt samples was determined by the centrifugation of 5 g at 4500 × g for 15 min at 4 °C. The WHC was calculated as follows:

$$WHC\ (\%) = (1 - W_1/W_2) \times 100 \quad (2)$$

where: W_1 = weight of whey after centrifugation, W_2 = yoghurt weight Barkallah *et al.* (2017) [21].

Rheological evaluation of yogurt samples

The rheological properties of yogurt samples were determined using a Modular Advanced Rheometer System (Thermo Haake Mars, Germany), equipped with a measuring system with titanium geometry plate (diameter 40 mm, gap 1 mm). The measurement temperature was 8°C. Before performing any analysis, the samples were allowed to rest for 10 minutes. The samples were submitted to different shear stresses in order to monitor their tension and viscosity parameters. For obtaining the flow curves, shear rates from 0.02 to 100 s^{-1} and from 100 to 0.02 s^{-1} were applied. Then, it was evaluated the variation of samples' viscosity as a function of shear rate, in the same analysis interval. Different models were adjusted to the viscosity curve of each sample, in order to determine which model fits best the rheological characteristics of the sample. Also, the viscosity of samples was measured as a function of time for 10 minutes at a constant shear rate of 100 s^{-1}, collecting 40 graphical points. For the assessment of the oscillatory rheological properties of the samples, there were conducted frequency dependency tests in the frequency range from 0.1 to 10 Hz. The parameters calculations were carried out using RheoWin 4 Data Manager software (version 4.20, Haake). Three determinations of each rheological test were conducted for each sample [22].

Sensory evaluation of yogurt samples

The sensory analysis of yogurt samples was conducted by 21 panellists aged 20 - 60 years according to method by SR 6345:1995 [23]. For each sensorial characteristic there were assigned points from 0 to 5 and a coefficient of importance (weighting factor) as follows: for appearance, color, consistency and smell $f_w = 0.5$, and for taste $f_w = 2$. The non-weighted average score ($S_{a/n\text{-}w}$) was calculated for each sensorial characteristic by adding the points given by the tasters to the arithmetic mean. The weighted average score for the yogurt samples was calculated by weight factors, multiplying each non-weighted average score of each sensory feature by the corresponding weight factor: $S_{a/w} = S_{a/n\text{-}w} \times f_w$. The total weighted average score was calculated by the sum of all weighted average scores corresponding to the sensory attributes of an analyzed sample. On the basis of the overall average score, the assessment of the qualitative level of the yogurt samples from a sensory point of view was performed on a scale from 0 to 20 points and compared to the standard [24].

Statistical analysis

The statistical analysis was done using Statistical Analysis System Program, version 16.0 (SPSS Inc., Chicago, IL, USA) at a significance level at $p < 0.05$. The measurements were performed in triplicate for each sample and results were expressed as mean value ± standard deviation.

RESULTS AND DISCUSSION

Yogurt samples were analyzed after 24 hours of storage under refrigeration conditions (4 ± 0.5 °C).

Physicochemical properties of yogurt samples

Physicochemical properties of yogurt samples are shown in Table 2. The results obtained shows all yoghurt samples with vegetable protein addition have lower *p*H values ($p < 0.05$) and a higher acidity ($p < 0.001$) than the control sample. One explanation for this aspect is that the plant proteins improve the growth of bacteria contained in the yogurt samples [1].

Table 2. *Physicochemical properties of the yogurt samples*

Sample	Characteristics			
	*p*H	Acidity [g acid lactic/100 g]	Syneresis [%]	WHC [%]
Y_C	4.58 ± 0.02	8.24 ± 0.1	44.4 ± 0.2	51.80 ± 0.1
Y_{NHP}	4.52 ± 0.01	9.43 ± 0.2	39.6 ± 0.2	59.16 ± 0.3
Y_{PSF}	4.50 ± 0.01	9.66 ± 0.1	49.8 ± 0.1	44.78 ± 0.1
Y_{PPI}	4.54 ± 0.03	9.39 ± 0.3	46.0 ± 0.3	58.96 ± 0.2
Y_{WG}	4.42± 0.02	10.49 ± 0.1	50.0 ± 0.1	47.45 ± 0.1
Y_{SPI}	4.43 ± 0.01	10.30 ± 0.1	43.6 ± 0.2	53.18 ± 0.2

In fermented milks like as yogurt expulsion of serum is considered a primary defect can make a product unacceptable to consumers [5]. The yogurt samples showed significant difference (p < 0.001) with respect to syneresis and WHC values. The best result in terms of syneresis it recorded the yogurt sample with the addition the yogurt sample with natural hemp protein (Y_{NHP}), followed by yogurt sample with soy protein isolate (Y_{SPI}). The addition of wheat gluten (Y_{WG}) increased syneresis compared the yogurt without plant protein (Y_C) by 12.6 %, while the addition of pumpkin seed flour (Y_{PSF}) reduced water holding capacity with 13.7 % compared the control sample (Y_C). Akin and Ozcan (2017) observed that the incorporation of the plant proteins which also contain fibers into yogurt improves viscosity and reduced serum separation levels, fact confirmed in our study by the yogurt sample with natural hemp protein (Y_{NHP}) and the yogurt sample with soy protein isolate (Y_{SPI}) [1].

Rheological characterization of yogurt samples

Independent of the added plant protein, all yogurt samples showed non-Newtonian thixotropic behavior with flow. From the graphical representation of flow curves (Figure 1), it can be observed the thixotropic characteristics of samples, due to differences between rising and descending curve, as a result of breakage of the gel. These differences can be quantified as the area between the flow curves: the larger the area is, the higher is the thixotropic effect and the gel breakage is more pronounced. The analysis reveals that there were not significant differences between samples with different plant protein additions. However, it has been observed an exception, the yogurt sample with added wheat gluten (WG) had the largest area thixotropy.

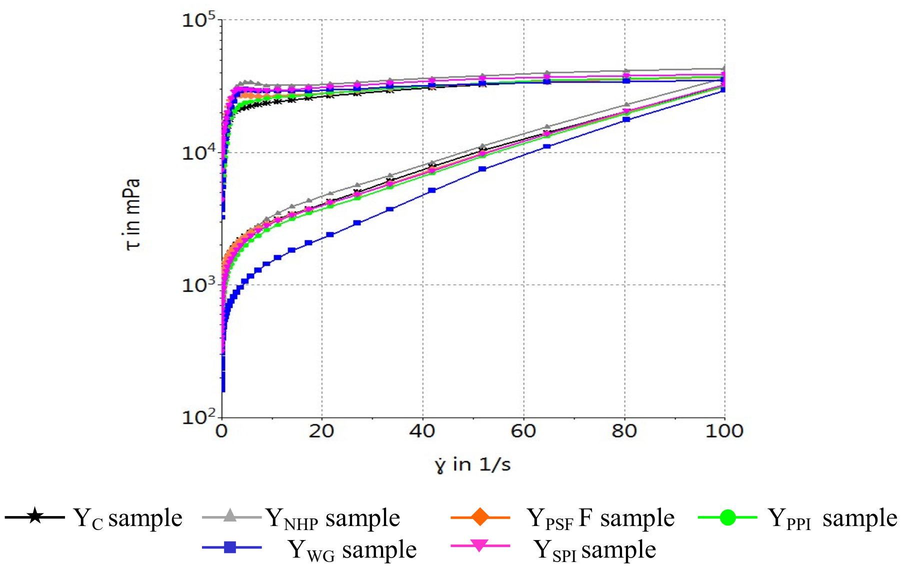

Figure 1. *Flow curves of yogurt samples*

Figure 2 shows the evolution of samples' viscosity as a function of time at a constant shear rate. Initially, the sample with natural hemp protein (Y_{NHP}) presented the highest viscosity value, followed by, in order, the sample with soy protein isolate (Y_{SPI}), the sample with wheat gluten (Y_{WG}), the sample with pea protein isolate (Y_{PPI}), the sample with pumpkin seed flour (Y_{PSF}) and, finally, the control sample. However, after submitting the yogurts for ten minutes to a constant shear rate of 100 s^{-1} the order of samples' viscosity was modified ($Y_{NHP} > Y_{PPI} > Y_C > Y_{PSF} > Y_{WG} > Y_{SPI}$), although the yogurt with hemp protein addition maintained the highest viscosity level.

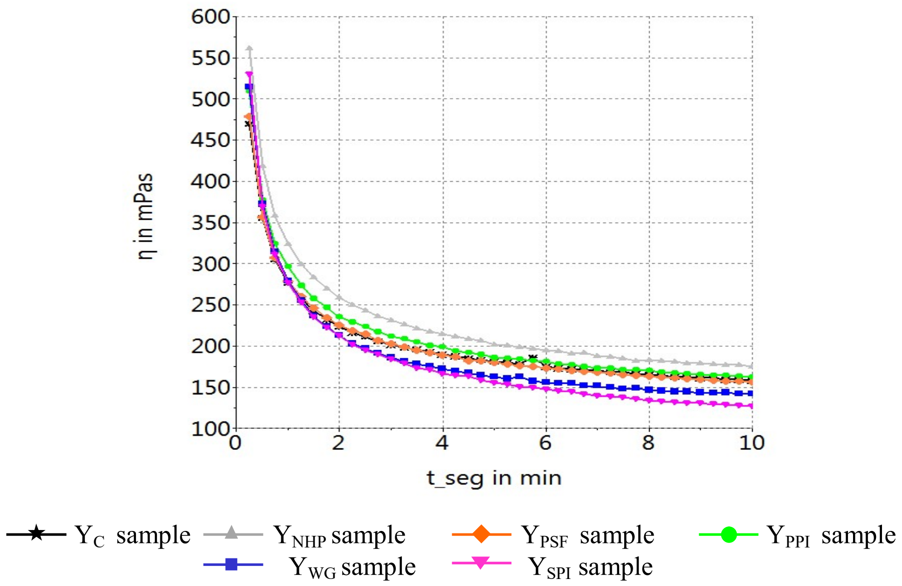

Figure 2. *Viscosity curves of yogurt samples*

The values of the regression coefficient *r* for models adjusted for the viscosity curves are given in Table 3. The best results were for the Herschel- Bulkley model (*r* between 0.9819 and 0.9981), whose calculated parameters (see Equation 3) for each sample are shown in Table 4.

$$\eta = {}^{\tau_0}/_{\gamma} + K\gamma^{n-1} \tag{3}$$

where: η = viscosity ($Pa \times s$); τ_0 = yield stress; γ = shear rate (s^{-1}); K = consistency index ($Pa.s^n$); n = behavior index (*dimensionless*).

Table 3. *Rheological models adjusted to the viscosity curves*

Sample	Bingham	Ostwald-De Waele	Herschel-Bulkley
Y_C	0.9898	0.9933	0.9900
Y_{NHP}	0.9710	0.9828	0.9850
Y_{PSF}	0.9946	0.9951	0.9956
Y_{PPI}	0.9858	0.9927	0.9935
Y_{WG}	0.9929	0.9959	0.9981
Y_{SPI}	0.9379	0.9720	0.9819

According to the Herschel-Bulkley model, there were calculated the rheological parameters, τ_0 *(Pa)*, *K* ($Pa \times s^n$), *n (dimensionless)* and $\eta_{-}(Pa \times s)$, for each sample, which are shown in Table 2. Viscosity at shear rate of ~ 50 s^{-1} has been suggested to have a good correlation with perceived thickness, stickiness and sliminess for a wide range of food products from Newtonian fluid to thick emulsion [22, 24]. The apparent viscosity

$\eta_{51,94}(Pa^* s)$ at the shear rate of 51,94 s^{-1} was 0.6247 Pa^*s for the control sample and 0.7303 Pa^*s for NPH sample. At high concentrations of protein, the casein aggregates may be trapped within the increasingly viscous solution explaining the big increase in apparent viscosity.

Table 4. *Herschel-Bulkley model parameters*

Sample	τ_0 (Pa)	K (Pa*s^n)	n	$\eta_{51.94}$ (Pa*s)
Y_C	-85.06	99.08	0.01516	0.6247
Y_{NHP}	-123.3	137.1	0.01566	0.7303
Y_{PSF}	5.730	7.621	0.6723	0.6354
Y_{PPI}	-75.16	85.00	0.01610	0.6427
Y_{WG}	2.686	8.864	0.6549	0.6309
Y_{SPI}	-219	240.4	0.01723	0.6906

Dynamic oscillatory shear test can be used to determine the viscoelastic properties of yogurt samples with the addition of vegetable protein. Dynamic mechanical spectra (G', G'' and η^*) as a function of frequency (f) of yogurt samples were modelled as an exponential function of angular frequency (ω), (see Equation 4 and 5):

$$G' = K' * (\omega)^{n'} \tag{4}$$

$$G'' = K'' * (\omega)^{n''} \tag{5}$$

where K' $(Pa*s^{n'})$ and K'' $(Pa*s^{n''})$ are constants; n' and n'' may be referred to as the frequency exponents; ω $(rad*s^{-1})$ is the angular frequency.

The values of dynamic oscillatory shear test parameters at frequency of 1 Hz are shown in Table 5. For evaluating the relative effects of viscous and elastic components in the viscoelastic behaviour of samples, the loss tangent ($tan\ \delta$) was calculated according to the Equation 6:

$$\tan\delta = \frac{G'}{G''} \tag{6}$$

Table 5. *Values of dynamic oscillatory shear test parameters at frequency of 1 Hz*

Sample	tan δ	$\lvert\eta^*\rvert$	$\lvert G^*\rvert$
Y_C	0.2685	24.23	152.2
Y_{NHP}	0.2983	36.20	227.4
Y_{PSF}	0.2823	26.83	168.6
Y_{PPI}	0.2967	11.94	75.0
Y_{WG}	0.2892	20.48	128.7
Y_{SPI}	0.2889	18.51	116.3

For describing the total resistance to deformation of samples, the complex modulus G^* *(Pa)*, and the complex dynamic viscosity, (η^*) were used. These parameters are indicator of the overall response of sample against the sinusoidal strain calculated by Equations 7 and 8.

$$G^* = \sqrt{(G')^2 + (G'')^2} \tag{7}$$

$$\eta^* = \frac{G'}{\omega} \tag{8}$$

The complex viscosity, $|\eta^*|$, at the 1 Hz frequency, was between 11.94 Pa*s for PPI sample and 36.20 Pa*s for NHP sample. Loss tangent (*tan δ*) was comprised between 0.2685 (Control sample) and 0.2983 (NHP sample).

Sensory characterization of yogurt samples

Sensory analysis is one of the most extensively used tools for quality characterization, as it allows correlations with other kind of parameter, such as rheological or physicochemical data [8]. Results evaluation by sensory analysis showed no significant differences on the acceptance of the samples prepared with plant protein and control samples.

The final results of the sensorial evaluation of the yogurt samples are shown in Figure 3. Yogurt samples with the highest acceptance scores, 19.2 were yogurt with pumpkin seed flour (Y_{PSF}), followed by yogurt with natural hemp protein (Y_{NHP}), 18.6.

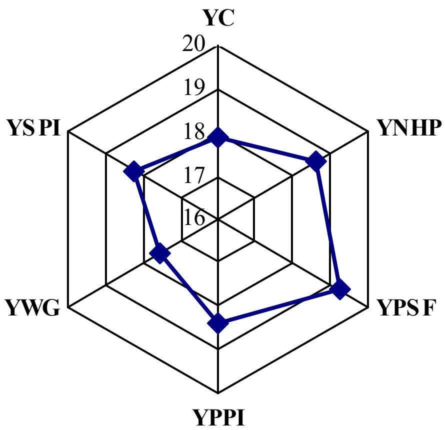

Figure 3. *Graphical representation of total weighted average score of yogurt samples*

The relationship between characteristics of the yogurt samples and between the yogurt samples obtained

PCA loadings of the physicochemical, rheological and sensorial values of the yogurt samples are shown in Figure 4. The two plots represented 62.10 % and 37.74 % of the total variance. The first principal component PC1 underlines a close association between the yogurt rheological parameters complex viscosity and complex modulus and opposes the sensorial score and syneresis values which are inversely correlated (r = -0.571). The second main component opposes the acidity and pH values which are significant correlated (r = -0.959) at $p < 0.01$ and the parameter syneresis and WHC between which there is a negative correlation (r = - 0.796) at $p < 0.05$.

The correlation obtained between the results of the yogurt samples are shown in Figure 5 and Table 6. The first two principal components explain 99.25 % of the total variance (PC1 = 97.42 % and PC2 =1.83 %). In respect to the first principal component PC1, one can notice that there is a very good correlation between the yogurt samples Y_C, Y_{NHP}, Y_{PSF}, Y_{WG} and Y_{SPI}. These yogurt samples are strongly associated with the first component PC1. The second principal component, PC2 is strongly correlated to the quality of Y_{NHP} yogurt which is characterized by the highest values of WHT, complex viscosity and complex modulus from the yogurt samples. However, good correlation has been obtained between all the yogurt samples analyzed as have seen from the Table 6 and Figure 5 were all the samples are placed above the PC axis on the right sight of the PCA graph.

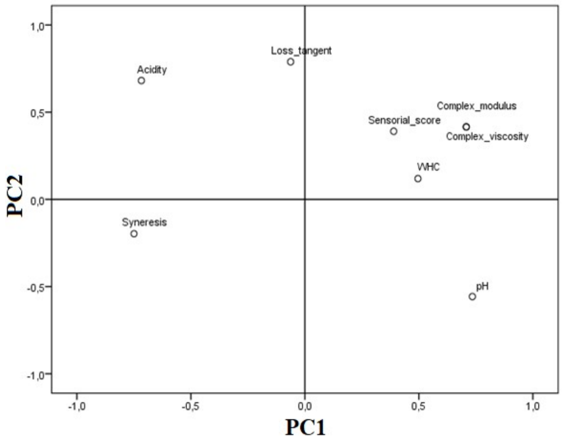

Figure 4. *Loading plot of first two principal components based on physicochemical, rheological and sensorial properties of the yogurt samples*

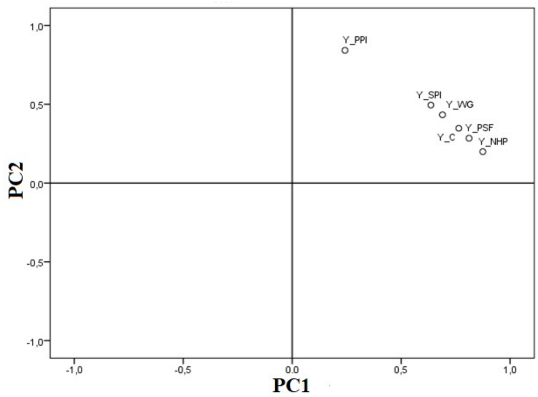

Figure 5. *Principal component analysis of the yogurt samples characteristics*

Table 6. *Matrix of correlation of yogurt samples*

Variables	Y_C	Y_NHP	Y_PSF	Y_PPI	Y_WG	Y_SPI
Y_C	1	0.991**	0.996**	0.882**	0.995**	0.991**
Y_{NHP}	0.991**	1	0.992**	0.818*	0.974**	0.967**
Y_{PSF}	0.996**	0.992**	1	0.850*	0.991**	0.979**
Y_{PPI}	0.882**	0.818*	0.850*	1	0.909**	0.937**
Y_{WG}	0.995**	0.974**	0.991**	0.909**	1	0.995**
Y_{SPI}	0.991**	0.967**	0.979**	0.937**	0.995**	1

* Correlation is significant at a level of $p < 0.05$
** Correlation is significant at a level of $p < 0.01$

CONCLUSIONS

The results of this study showed that it is possible to produce a yogurt with the addition of vegetable proteins. The addition of natural hemp protein (NHP) and pumpkin seed flour (PSF), two raw materials that have not been investigated in the production of yogurt have led to consumer acceptance of finished products, and the good results of rheological and physicochemical evaluation.

Generally, the addition of plant proteins in the production of yogurt can improve the nutritional value respectively physicochemical, rheological and sensory properties of finished product. This plant proteins are not affected the fermentation and increasing protein concentration in finished product.

In conclusion the vegetables are could be potentially as a source of protein can be useful in new product development.

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