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USE OF EXPERIMENTAL DESIGN FOR QUALITY OPTIMISATION OF YOGURT WITH ROSEHIP POWDER AND GRAPE SEED EXTRACTS

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ABSTRACT

Worldwide, the yogurt is a very popular fermented dairy food and it is known for its health benefits, nutritional value and digestibility. The factors that influence the quality of yogurt are milk composition, dry matter content, the temperature and time at which pre-treatment of the milk is carried out, the type and the quantity of starter culture used, incubation temperature and storage conditions for the final product. A major problem in the production of yogurt is the whey separation. In the yogurt industry, syneresis is considered as a defect for the yogurt quality which cannot be accepted by consumers. This paper proposes the use of two natural ingredients to improve the yogurt quality, especially to reduce the separation of whey. The addition of rosehip powder and grape seed extract in the yogurt formula leads to the modification of its physicochemical and sensory properties. The results show the possibility of producing yogurts containing these ingredients without changing the technological procedure and with a good quality for the consumers. Yogurt with the best physicochemical and sensory properties was obtained by adding 1.5% rosehip powder and 0.5% grape seed extract to its manufacturing formulation.

Keywords: yogurt, rosehip powder, grape seed extract, syneresis

INTRODUCTION

Lately, the interest of the consumers in certain bioactive food nutrients with positive role on human health has increased [1]. Also, the popularity of yogurt as a functional food has expanded significantly. Therefore, yogurt is well known as a conventional food with therapeutic, nutritional and sensory properties. In recent years, it has become of great importance, in terms of food safety, the use of natural ingredients, instead of artificial supplements [2]. Alternatives to synthetic preservatives are the antioxidants present in plants, algae and mushrooms, which are excellent natural additives [3].

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The use of rosehip fruits as a vitamin supplement or in health food products is very common in several countries from Europe, especially due their high content in ascorbic acid [4] and also for their rich composition in minerals (K, P) and vitamins [5]. It is well known the strength effect of rosehip in the body defence mechanisms against infections, especially common cold [6]. The results of a recent study showed the rosehip potential to inhibit cancer cell proliferation due to its high content in phenolic compounds. Therefore, the rosehip antioxidant capacity is strictly related to its anticancer effect [7]. Rosehip extracts also possess antimutagenic effects [8]. The spontaneous flora of Romania includes the rosehip plants. The rosehip growth area in Romania spreads from the Black Sea coast to the mountain regions at the altitude of 1200 m, on meadows, on rides with southern exposure, on the railways, in parks on the roads and, also, in the ravines of the deciduous forests or on their edges. In comparison with the other fruits and vegetables, rosehips have the highest vitamin C content (30–1300 mg/100g). In addition, rosehips contain carotenoids, mainly lycopene, β-carotene, and only traces of lutein and zeaxanthin [9]. Therefore, rosehip is a fruit preferred by many consumers who want to maintain a healthy diet, especially due to the high vitamin C content and excellent taste.

Grape (*Vitis vinifera* L.) is one of the world's largest fruit crops. The solid by-products resulting from the processing of the fresh grapes, principally from winemaking industry, represent approximately 13% of the total grapes weight. The winemaking technologies generate the by-product which is commonly called grape pomace, composed of seeds and skins. Grape pomace contains fibres, oil, proteins, minerals and complex phenols [10]. Grape seeds have a high content of polyphenolic compounds which have been associated with increased antioxidant properties. The antioxidants extracted from grape seeds are a safer alternative to synthetic antioxidants and include several polyphenol components: anthocyanins, proanthocyanidins, catechins and flavanols [11]. Because of the high antioxidant activity of these compounds, the grape pomace by-product has health-promoting potential and disease protective effects. On these grounds, grape pomace has gained the attention of researchers as novel ingredients or food additives, which can bring extra health benefits to various food products and, at the same time, could be a solution for the waste disposal problem. Only in Europe, the annual production of grape by-products, such as skins, seeds and stems, is estimated at 14.5 million tonnes [2]. As the disposal fees and fines for unauthorized discharges in industries have increased considerably, the recovery/reuse of by-products is of economic concern nowadays. The most common use is in obtaining extracts, products which have been proposed to be applied by the food industry [12]. Grape seeds extract contains high concentrations of bioactive compounds and was used as an additive in dairy products, in different amounts [13, 14]. Several studies emphasized that the grape seeds extract reduced oxidation and provided higher microbial stability in foods, having no significant influence on the sensory characteristics that can affect the consumer's decision to purchase a food product [13, 14].

However, prior to industrial production of yogurt with rosehip powder (RP) and grape seeds extract (GSE) addition, there must be performed laboratory researches in order to determine to what extent the incorporation of RP and GSE in the milk influences the yogurt quality. Thus, the aim of this study was to evaluate the physicochemical and sensory properties of yogurt made with varying RP and GSE levels. Also, it was determined the effect of RP, GSE and mixture interaction on the properties of yogurt and it was obtained the best formulation to produce a high quality finished product.

MATERIALS AND METHODS

The Design-Expert (version 7.0.0) software was used to determine the optimum doses of the rosehip powder and grape seed extract in yogurt formulation. A D-optimal mixture design was used with the constraints rosehip powder (x_1 or RP) + grape seed extract (x_2 or GSE) = 2% as expressed in Table 1. According to D-optimal approach the effect of these components on yogurt quality parameters in terms of physicochemical and sensorial characteristics was evaluated and the optimum combination was determined. For optimum formulation, depending on the influence of each factor, rosehip powder and grape seed extract, it was determined the combination of factors that led to the best responses concerning quality properties of yogurt as finished product.

Table 1. Coded and real value of formulation factors used in D-optimal mixture design

Run	Coded values		Real values	
	RP	*GSE*	*RP* (%)	*GSE* (%)
1	0.000	1.000	0.000	2.000
2	0.126	0.874	0.250	1.750
3	0.252	0.748	0.500	1.500
4	0.375	0.625	0.750	1.250
5	0.500	0.500	1.000	1.000
6	0.625	0.375	1.250	0.750
7	0.748	0.252	1.500	0.500
8	0.625	0.375	1.250	0.750
9	1.000	0.000	2.000	0.000

RP, rosehip powder; GSE, grape seed extract.

The technological process for obtaining this type of yogurt complies with the steps of the conventional technology. It was used raw cow's milk with following characteristics: 3.5% fat content, 3.0% protein and 4.5% carbohydrates. Rosehip powder and grape seed extract were added in yogurt formulation in different percentages (0÷2%) (Table 1). The ingredients mixture was pasteurized at 90°C for 15 min, and then cooled to 42°C. For inoculation, it was used the yogurt starter culture DI–PROX (Enzymes & Derivates, Romania), composed of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The samples were inoculated with 0.2% (w/v) yogurt starter culture and incubated at 42 ± 0.2ºC, until the pH reached 4.6 ± 0.2. The yogurt samples were stored at 4.0 ± 0.5°C for 24h, and then the analyses were performed in order to determine the physicochemical and sensorial properties. All samples were prepared in triplicate.

The yogurt samples were analysed for pH, titratable acidity, syneresis, colour parameters and sensory properties. The used equipment respect the requirements in the standard methods. The monitoring of pH was conducted using a F2 Standard Mettler Toledo (Germany) portable device according to STAS 8201-82. The titratable acidity determination was made by respecting the following classical method: a blend of 10 mL of yogurt samples, 20 mL distilled water and 3 drops of phenolphthalein were titrated with NaOH 0.1N until the stabilization of a pale pink colour. The results were expressed as Thörner degree. For the syneresis determination there were used 10 mL of yogurt which were weighed. Yogurt samples were placed in tubes of Spin MPW 223E Centrifuge (Germany) and centrifuged at 639 × g for 10 min, at 4±0.5°C. The clear supernatant was poured off, weighed and expressed as percent weight relative to original weight of yogurt, according to the Eq. (1) [15].

$$\text{Syneresis (\%)} = (\text{Weight of supernatant} / \text{Weight of yogurt sample}) \times 100 \qquad (1)$$

Colour parameters have been determined using a Konica CR400 Chromameter (Konica Minolta, Japan). The colour intensity, hue angle and ΔE* were calculated by Eqs. (2) – (4):

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \qquad (2)$$

$$h^*_{ab} = tan^{-1}\left(\frac{b^*}{a^*}\right) \qquad (3)$$

$$\Delta E^* = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad (4)$$

, where L* (100 = white; 0 = black), a* (+ red, - green), b* (+ yellow; - blue), C_{ab}^* - chroma, h_{ab}^* - hue angle, and ΔE* - colour difference. All analyses were performed in triplicate.

The sensory analysis of yogurt samples was conducted by 33 panellists aged 20-60 years according to method by SR 6345:1995. For each sensorial characteristic there were assigned points from 0 to 5 and a coefficient of importance (weighting factor) as follows: for appearance, colour, 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 analysed 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, as described in SR 3665-1999 [15].

The relationship between the responses and factors was established using the model for D-optimal which is based on the quadratic model in Scheffé equation (Eq.5):

$$E = \sum_{i=1}^{2} \beta_i \cdot x_i + \sum \sum_{i \langle j}^{2} \beta_{ij} \cdot x_i \cdot x_j + \sum_{i=1}^{2} \beta_{ii} \cdot x_{ii}^2 \qquad (5)$$

, where E is the predictive dependent variable such as physicochemical evaluation parameters (pH, acidity, syneresis), colour parameters (L^*, a^* and b^*) and overall average score for sensory characteristics. β_i, β_{ij} are coefficients for linear and non-linear terms, and X_i is factors proportion in terms of real-components. An analysis of variance (ANOVA) at $p < 0.05$ was conducted for each of the response variables, testing the value of the applied model and determining if a more complex model could have a better fit.

RESULTS AND DISCUSSION

The best model was fitted according to following statistical parameters: coefficient of determination (R^2), adjusted coefficient of determination (*Adj-R^2*), predicted coefficient of determination (*Pred-R^2*) and p-value. *P*-values of the acceptable model for each response, obtained by the analyses of variance, were lower than 0.05. The models were significant for the response variables with a R^2 which varied from 0.69 to 0.99 (Table 2). For syneresis characteristic no significant models were obtained.

Table 2. Regression coefficients and correlation for the adjusted model to experimental data in D-optimal mixture design for physicochemical properties, colour parameters and sensory analysis

Variable	β_1 (RP)	β_2 (GSE)	$\beta_1 \cdot \beta_2$	$\beta_1 \cdot \beta_2 \cdot (\beta_1 - \beta_2)$	R^2	*Adj-* R^2	*Pred-* R^2
Physicochemical parameters							
pH	4.52^{b}	4.59^{b}	-0.18^{b}	-	0.89	0.85	0.65
Acidity (°T)	85.47^{a}	78.90^{a}	32.89^{b}	-	0.77	0.70	0.42
Syneresis (%)	47.11^{ns}	43.64^{ns}	7.71^{ns}	-32.48^{a}	0.69	0.50	- 2.31
Colour parameters							
L^*	77.56^{b}	68.74^{b}	-6.75^{b}		0.99	0.98	0.98
a^*	-0.54^{b}	3.48^{b}	2.44^{b}	-	0.99	0.99	0.99
b^*	21.08^{b}	18.09^{b}	-2.67^{b}		0.98	0.98	0.97
Sensory analysis							
Overall average score	18.43^{b}	18.02^{b}	3.26^{b}	3.46^{a}	0.90	0.85	0.67

[a] Significant at 0.05 level; [b] Significant at 0.01 level; [ns] Non significant at 0.05 level.

The quadratic model obtained for pH was highly significant to the obtained data ($p<0.01$) and was fitted for describing the relationships between the factors and responses. Effect of each factor and interaction between them showed that RP and GSE factors increased pH value and their combinations decreased it. The results revealed by the present study are in accordance with the results reported by Chouchouli *et al.* (2013) and Marchiani *et al.* (2016).

According to Table 2 quadratic was the best model for acidity parameter. Linear terms and two component combination showed positive coefficients for acidity parameter. The model obtained showed that the most significant effect on acidity value has RP and GSE interaction ($p < 0.01$). An increase in acidity with the increase of RP and GSE proportion in yogurt sample can be due to the compounds from RP and GSE.

Experimental modelling results for syneresis are shown in Table 2. It can be seen that linear terms and RP-GSE interaction are not significant effects on syneresis. A negative *Pred-R^2* implies that the overall mean is a better predictor of syneresis parameter than the quadratic model.

As shown in Table 2, RP and GSE increased significantly ($p < 0.01$) the lightness of colour, while RP-GSE interaction had a significant ($p < 0.01$) negative effect on L^*. The quadratic model for L^* colour parameter obtained was significant at level of $p = 0.01$.

Addition of GSE to yogurt formulation showed a positive coefficient, indicating that GSE had a positive effect on a^* colour parameter. Also, interaction RP/GSE showed a positive coefficient indicating that rosehip powder had a synergistic effect on a^* parameter. Study on the effect of RP on a^* showed that this factor produced a negative effect at significance level ($p < 0.01$).

As regards the b^* colour parameter, the obtained quadratic model was significant at level of $p < 0.0001$. The RP and GSE addition in yogurt formulation have a significant effect ($p < 0.01$), increasing b^*, but RP/GSE interaction showed negative coefficient for b^*.

The analysis of the model obtained for overall average score revealed that both linear terms and RP-GSE interaction showed positive coefficients (Table 2) which indicated increasing this parameter. Each factor and interaction between them showed significant effect ($p < 0.01$) on overall average score.

Optimum formulation was obtained based on minimum pH and syneresis, in range acidity and colour parameters, and maximum overall average score. Using this approach a set of combination of rosehip powder and grape seed extract was found. This yogurt formulation with natural ingredients as rosehip powder and grape seed extract and predicted properties are shown in Figure 1. Desirability of these proportions, obtaining according to the desirability of pH, acidity, syneresis, L^*, a^*, b^* colour parameters and overall average score was found 0.854. The value obtained for desirability (> 0.800) indicates that the yoghurt quality was regarded to be acceptable. The yogurt control sample presents the following characteristics: pH = 4.48, acidity = 101°T, syneresis = 53.11 %, L^* = 86.44, a^* = - 7.12, b^* =13.64, overall average score =14.40.

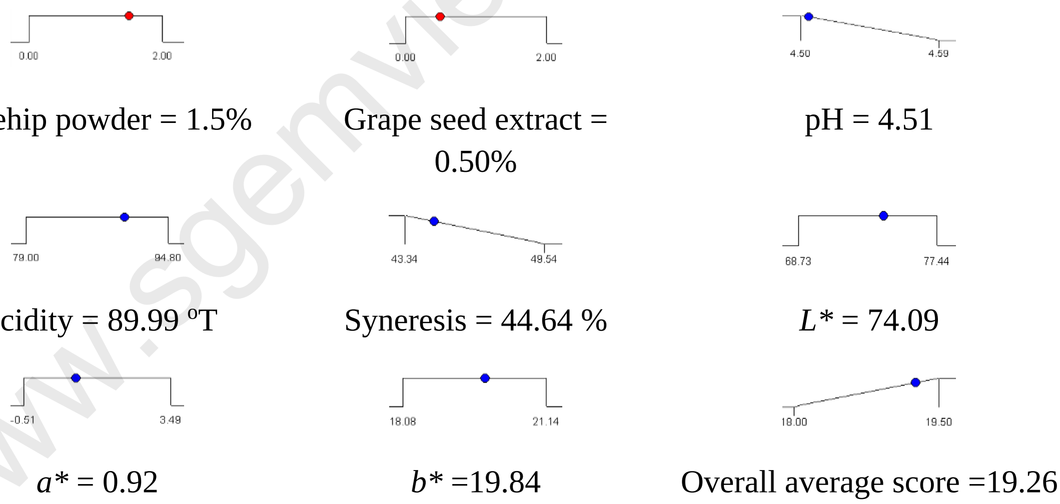

Figure 1. Desirability ramp

The values of acidity, syneresis and L^* colour parameter were higher compared to the optimum sample formulated, indicating the fact that the yogurt with 1.5% rosehip powder and 0.50% grape seed extract is improved from of quality characteristics point of view.

CONCLUSION

The addition of rosehip powder and grape seed extract in the yogurt formula leads to the modification of its physicochemical and sensory properties. Incorporation of rosehip powder and grape seed extract changed the quality characteristics of yogurt in different ways, which is why this research employed a statistical approach by using D-optimal mixture design.
The best model, based on the results of pH test, acidity test, *L**, *a**, and b* colour parameters test, was found to be the quadratic model. However, for syneresis test and sensory analysis overall average score, the cubic model has been chosen. The optimal composition of the yogurt formulation was obtained based on the each desired response. Yogurt with the best physicochemical and sensory properties was obtained by adding 1.5% rosehip powder and 0.5% grape seed extract to its manufacturing formulation.
The results showed that production of yogurt with rosehip powder and grape seed extract combination is feasible and that it is possible to produce yogurts containing these ingredients without changing the technological procedure and with a good quality for the consumers.

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