Sodium reduced bread as a delivery vehicle for dietary prebiotics to enhance food functionality

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Abstract - Inulin, obtained from chicory, was added at different concentrations (from 2 to 10% on flour basis) to enhance the food functionality of wheat bread. Flour samples were analysed regarding: gluten content (%); protein content (%); gluten deformation (mm); ash content (% d.w.) and rheological behaviour. It was found that the physico-chemical properties of the enriched flour remained similar to those of the control. Rheological properties of the dough, according to the farinograph tests were improved with the addition of inulin, resulting in increase of development time, stability and quality and the decrease of softening. The Consumer Acceptance Test established the optimum amount of inulin (4% addition) in the flour to complete the first stage of functional product development. The second stage continued with replacement of NaCl in bread enriched with 4% inulin with preselected miner

Key words — bread quality, functional food, prebiotics, sensory analysis, simplex centrioid design, sodium reduced, wheat bread.

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There is an evidence that prebiotics can impart a range of health benefits if consumed on a regular basis. There is a number of papers and reviews on the topic of prebiotics and their health benefits [1], [2]. The health benefits include increased mineral absorption [3], [4] improved immune response [5], [6], colorectal cancer prevention [7], cancer therapy [8] as well as their role in feelings of satiety and weight management [9].

In recent years, there has been a clear trend in increasing the number of patents on low-salt foods. Different approaches were proposed, most of them based on replacement with verious salts and addition of different substances to preserve the sensory quality [10], [11], [12], [13], [14]. Low sodium salt mixtudes are consisting of sodium chloride and other salts such as chlorides, carbonates or sulphates together with one or more aromatic additives to mask the bitter or metallic aftertaste [15]. Changing ordinary sodium salt with low sodium, high potassium, high magnesium content is a valuable non-pharmacological approach to lowering blood pressure in patients suffering mild to moderate hypertension [16].

The aim of this project is to develop a sodium-reduced food, which is characterized by daily consumption, e.g. bread, with enhanced functionality targeted to the general population.

2 MATERIALS AND METHODOLOGY

2.1 Materials of experiments

Eight components were prepared to formulate dough of wheat bread that contained inulin: wheat flour with moisture content (13.8 %), gluten content (26 %); gluten deformation (1.5 mm); ash content (0.42 % d.w.); inulin "Fibruline Instant"; water, EN 806-1:2003 [17]; yeast (BDS 483 – 90); salt (PMS № 23/ 2001) [18]; margarin (40% fat); yougurt (1.5% fat) and vinegar.

Mineral salts used for NaCL substitution were chemically pure with a purity above 99.0% as follows:

• $MgCl_2$ and KCl - according to regulation No 21 of 15 October 2002 [19]. on the specific criteria and requirements for the purity of additives intended for use in food.

2.2 Methods

Bread dough was prepared according to the *Baking method* developed in UFT, Plovdiv [20] consisting of white flour 100 g, water 40.0 x 10^{-3} m^3; salt 1.5 g, yeast 3.0 g, yougurt 10 g, margarin 1.0 g, vinegar 0,7 x 10^{-3} m^3. Inulin of different concentrations [2 g (B), 4 g (C), 6 g (D), 8 g (E) and 10 g (F), flour basis] was added to the control (K). The rheological properties of the dough enriched with fibers was measured by pharinograph (Brabender AT Model 50). The dough was prepared at temperature (34.5 ± 0.5^{0}C), resting time (20 min), bread formation, final fermentation (30.0 ± 0.5^{0}C), and baking temperature (230^{0}C). Specific volume was measured in (cc/ g) which are the ratio of volume to the mass of bread as well as index (H/D) of bread (the ratio804 of height to diameter of bread). A consumer panel (n=33) evaluated overall acceptability and seven other sensory attributes (appearance, aroma, flavor, texture, sweetness, acidtty and aftertaste) of six treatments (K, B, C, D, E, F). Participants evaluated samples using environmentally-controlled partitioned booths illuminated with white incandescent light in the sensory lab at the Institute of Preservation and Food Quality (IPFQ). Experiments were replicated twice in a randomized block design. A nine-point hedonic scale was used for samples evaluation.

In this research a three-component, constrained simplex centrioid mixture design was applied to establish the optimum concentrates of KCl and $MgCl_2$ for salt partial or complete substitution in the inulin enriched bread basic formulation. The mixture components consisted of NaCl (X_1), KCl (X_2) and $MgCl_2$ (X_3). Component proportions were expressed as fractions of the mixture with a sum ($X_1 + X_2 + X_3$) of one (Table 3). The ten points were three single ingredient treatments, three two-ingredient mixtures and four three-ingredient mixtures. In order to optimize the multicomponent mix, the overall sensory acceptance, the specific volume (cc/ g), the H/D index and the firmness of the crumb (H, kg) of the samples were selected as the target functions. The bread firmness was measured on a minimum of 5 slices per sample using TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., England).

Scheffe's canonical special cubic equation for three components was fitted to data collected at each experimental point using backward stepwise multiple regression analysis as described by Cornell [21]. The postulated canonical special cubic equation was:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (1)$$

where Y is a predictive dependent variable (specific volume - V, H/D index - F, firmness - H, and overall acceptance - SV); β_1, β_2, β_3, β_{12}, β_{13}, β_{23}, and β_{123} are the corresponding parameter estimates for each linear and cross-product term produced for the prediction models for NaCl, KCl, and $MgCl_2$, respectively. An analysis of variance was performed on the data and response surfaces were generated for each response using predictive models. The fitted model for V, F, H, and SV was used to optimize the salt reduction in the inulin enriched bread basic formulation. The terms in the canonical mixture polynomial have simple interpretations which can be found in specialized texts [22]. The usual way to summarize mixture proportions is via triangular (ternary) graphs. The value for the dependent variable can be indicated in a two-dimensional plot where the contour of constant height is graphed on a triangle.

3

The influence of soluble fiber (inulin) on the rheological properties of the dough was measured by farinograph. The following rheological parameters of wheat flour with /without added inuline were found: water absorbtion capacity, development time, and stability, degree of softening after 10 and 12 min exposure (Table 1).

Table 1. Rheological parameters of wheat flour with /without added inuline

Rheological indices	K	B	C	D	E	F
Water absorption capacity of flour (for 500 UB) %	60.5	60.2	52.9	54.7	52.1	50.1
Dough development time, min.	2.2	1.5	1.7	6.9	8.5	9.5
Stability, min.	3.0	4.7	5.8	9.0	11.2	12.2
Degree of softening, after 10 min. UB	73	73	78	34	10	4
Degree of softening, after 12 min. UB	135	89	101	0	0	0
Quality farinographic indices	40	38	35	98	117	129

Table 1 shows that the water absorption capacity of the dough decreases as the concentration of the inulin increases (2 to 10%). Dough development time and stability were greatly enhanced by the addition of 6, 8 and 10% inulin, which resulted in the dough structure strengthening. The incorporation of 2 and 4% inulin does lead to development time similar to the control and an increase in dough stability compared to the control. Şahin et al., 2011 [23] reported in their study on bread wheat the lowest farinograph water absorption value; 52.6, the highest value; 63.1 and significant positive correlation between farinograph water absorption and bread volume was determined. Other authors [24] found high water absorption, combined with low degree of softening indicates good quality flour, whereas a high water absorption combined with a high degree of softening indicates poor quality flour. From the results obtained it is obvious that inulin is well incorporated into the dough structure. The addition of inulin improves the rheological characteristics of the dough by increasing the dough development time and its stability, and decreasing the degree of softening.

Based on the method mentioned by Plovdiv, UFT, the comprehensive baking assessment of floor bread was made using baking laboratory test. Thus, describing standard indicators of floor type of bread can be obtained by evaluation the influence of inulin addition on the bread quality. Following baking laboratory test, measurements of specific volume (V) and H/D index of the bread samples was carried out (Figure 1& 2).

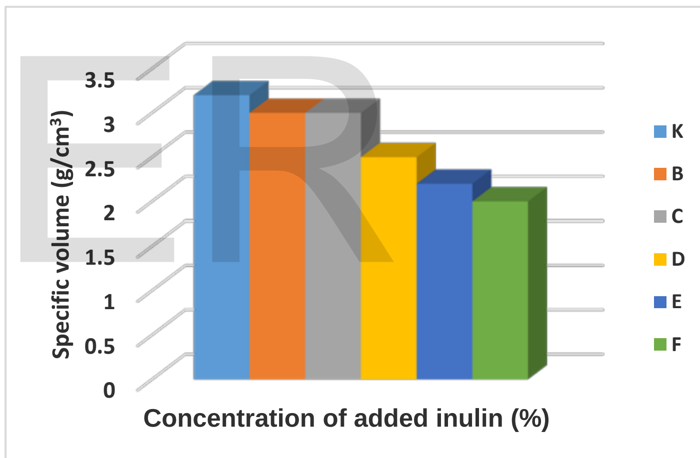

Fig. 1. Specific volume of bread entiched in fibers

Diagram on the specific volume of the floor breads shows reduction in the specific volume if compare to the control (K) for all the treatments. Results of samples B&C were the closest to the control sample (3.00 cm^3). The difference between values of the control sample K and the lowest performant sample F was (37.50 %). The index H/D is determined by the ratio between the height and the diameter of the bread after baking. Figure 2 showed the index H/D of the control and test samples of floor bread. Results of Figure 2 showed that the bread sample B, followed closely by C had the highest index H/D (0.40; 0,38, respectively) if compare to the control (0,44). Small deviations were noted in both samples – C and D, but they are practically insignificant (0.2 cm^3). With increasing the concentration of added inulin, index H/D decreased gradually.

The quality of the enriched bread is lower than that of the control which is probably due to the fact while inulin appears to integrate well to the gluten network, it also dilutes it resulting in lower gas retention ability [9].

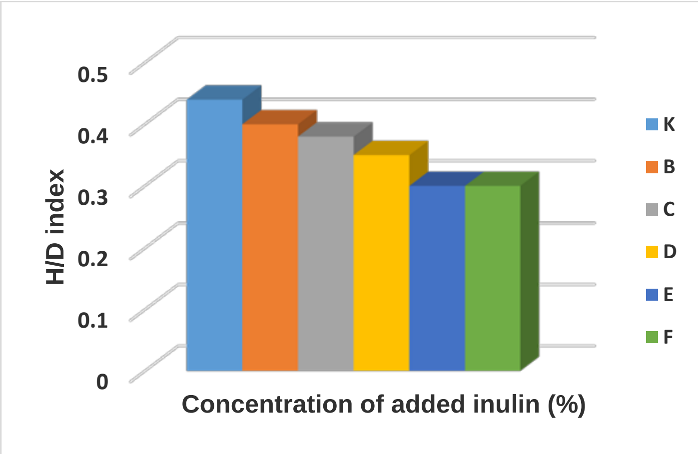

Fig. 2. H/D index of bread entiched in fibers

The baking laboratory test of floor bread was followed by a sensory test for acceptance.

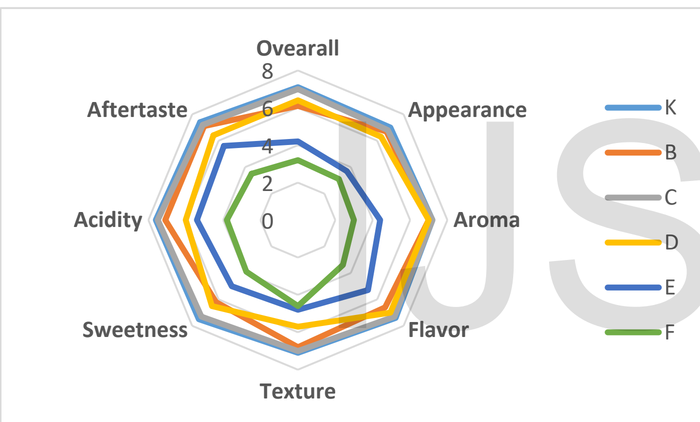

Fig 3. Mean hedonic ratings for overall acceptance, appearance, aroma, flavour, texture, sweetness, acidity and aftertaste for inulin enriched formulations and control.

Consumers' evaluated six samples of bread (K, B, C, D, E, F) presented monadically using the 9-point hedonic scale (dislike extremely, neither like nor dislike, like extremely). Mean values of "overall acceptance", "appearance", "aroma", "flavour", "texture", "sweetness", "acidity" and "aftertaste" of five bread samples enriched with inulin and the control are shown in Figure 3. Samples B , C and D (2, 4, 6% inulin) were scored ≥6 (slightly like) and similar to the control for "overall acceptance", "appearance", "aroma" and "flavour" (p=0.05). Bread with 4% supplement rated like the control on texture, sweetness and aftertaste followed closely by the 2 % sample (p=0.05). Samples with 2 and 4% were close to the control on "acidity". The rest of the samples (E and F) rated lower and significantly different than the control on all the sensory attributes (Table 2).

Table 2. The difference between means of hedonic ratings

Sam ples	Ovear all[a]	Appea rance[a]	Aro ma[a]	Fla vor[a]	Text ure[a]	Swe et[a]	Aci	After
K	d	d	d	d	c	d	d	de
B	d	d	d	d	cd	c	d	d
C	d	d	d	d	c	cd	d	de
D	d	d	d	d	ab	c	c	d
E	bc	bc	bc	bc	b	bc	bc	bc
F	a	a	a	a	ab	a	a	a

[a]Means in the same column not followed by the same letter are significantly different at p=0.05 as determined by t-paired means test

The consumer acceptance test showed the sensory attributes: "overall acceptance", "appearance", "aroma", "flavor", "texture", "sweetness", "acidity" and "aftertaste" were rated "like it moderately" (x≥7) for bread with 4% supplement, that was the closest to the control and fully met the consumers preferences. Based on the results from the sensory test the best performant formulation was chosen for further work.

The most difficult part of the reformulation of foods is obtainning a product equivalent in quality, texture and overall perception to the conventional ones.

In accord with the general objective of the study the formulation of optimal salt mixteres concentrates was established through simplex-centroid designs. Specifically, the study sought to perform an experiment to generate mixture data on KCl and $MgCl_2$ concentrates formulation to substitute NaCl in bread, fit the model for the three components simplex-centroid design, test the adequacy of the fitted model and determine the optimal salt concentrates. An experiment was done by blending salt concentrates using mixtures of NaCl, KCl and $MgCl_2$ guided by the design points of the simplex-centroid design.

Table 3 gives the matrix for conducting experimental work.

Table 3. Matrix for conducting the experiment

№	NaCl (X_1)	KCl (X_2)	$MgCl_2$ (X_3)
1	1	0	0
2	0	1	0
3	0	0	1
4	0,5	0,5	0
5	0,5	0	0,5
6	0	0,5	0,5
7	0,33	0,33	0,33
8	0,67	0,165	0,165
9	0,165	0,67	0,165
10	0,165	0,165	0,67

In pure technological aspect NaCl replacement in the bread recepie affects its quality characteristic including firmness of the crumb (moreover strong dough mixing properties are related to firm product texture) and sensory properties. Hence, the specific volume (cc/ g), the H/D index, the firmness (H, kg) of the samples and the overall sensory acceptance were selected as predictors.

Table 4 shows the specific volume (V) of the bread sample treatments.

Table 4. Mean values of specific volume of bread samples

Sample	Specific volume	Sample	Specific volume
1.	5.9a	6.	4.5c
2.	5.8a	7.	5.9a
3.	5.9a	8.	5.7a
4.	5.4ab	9.	5.5ab
5.	5.7a	10.	5.3ab

The ratio between the selected salts is shown in table. 3. Mean values denoted by the same letter are not statistically significant ($P < 0.05$).

The simplex method and related procedures of modelling and optimization were used to obtain an equation for the specific volume (Equation 2). The model adequacy was tested by first determining the significance of the model parameters resulting in high precision equation describing the change in the specific volume. Adjusted R^2 was then calculated to determine the fitness of the model. From the adequacy test, the model provided a good fit with adjusted R^2 of 0.94. Figure 4 shows the reflecting surface of the specific volume for the component mixture.

$$V = 5.87X_1 + 5.84X_2 + 5.85X_3 - 1.78X_1X_2 - 0.96X_1X_3 - 5.43X_2X_3 + 22.34X_1X_2X_3 \quad (2)$$

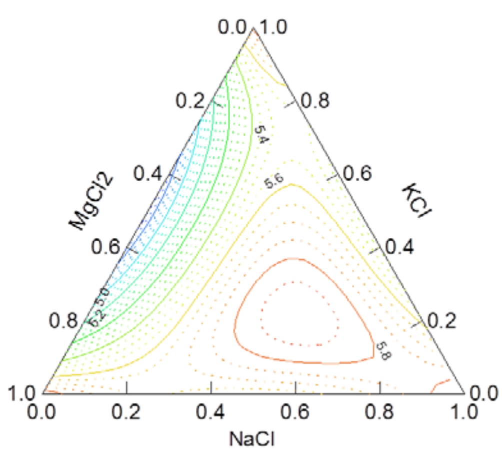

Fig. 4. Reflection surface of the specific volume of the component mixture

Highest specific volume values were observed for samples 1, 2, 3, 5, 7 and 8, and the lowest value was recorded for the sample without NaCl at the same ratio KCl - $MgCl_2$ ($P < 0.05$) (sample 6) (Table 4). Figure 4 shows the highest specific volume at the center and to the top of NaCl pinnacle, e.g. as the salt concentration increases, the specific volume also increases. The lowest specific volume is observed over the KCl-$MgCl_2$ area, and especially at the midle where the NaCl content is negligible, e.g. mixtures of the salts without NaCl have a negative effect on this parameter.

Table 5 shows the H/D index of the bread sample treatments.

Table 5. Mean values of H/D index of bread samples

Sample	H/D index	Sample	H/D index
1.	0.65a	6.	0.53ab
2.	0.50b	7.	0.58ab
3.	0.63a	8.	0.63a
4.	0.55ab	9.	0.48c
5.	0.54ab	10.	0.65a

The ratio between the selected salts is shown in table. 3. Mean values denoted by the same letter are not statistically significant ($P < 0.05$).

The mathematical model of the H/D index (F) of the component mixture (NaCl, KCl, $MgCl_2$) was obtained:

$$F = 0.66X_1 + 0.48X_2 + 0.65X_3 - 0.12X_1X_2 - 0.35X_1X_3 - 0.15X_2X_3 + 1.84X_1X_2X_3 \quad (3)$$

The model adequacy was tested and adjusted R^2 calculated to determine the fitness of the model. From the adequacy test, the model provided a good fit with adjusted R^2 of 0.78. Figure 5 shows the reflecting surface of the H/D index for the component mixture.

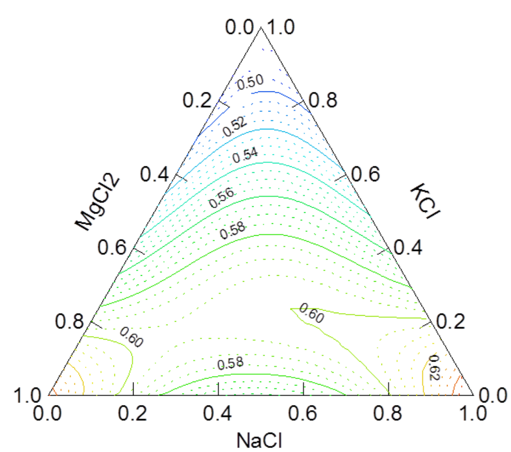

Fig. 5. Reflection surface of the H/D index (F) of the component mixture

The highest H/D index values were observed in samples 1, 3, 8, and 10, and the lowest value was noted in sample 9 ($p < 0.05$) (Table 5). Figure 5 shows the highest H/D index around the pinnacles of NaCl and $MgCl_2$, and the lowest H/D index was

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observed around the KCl peak, e.g. with an increase in the concentration of this salt in the mixtures the values of the index decreases. The experiments went on with measurement the firmness of the crumb. Figure 6 shows the mean values of the measured force (H, kg) and the corresponding standard deviations (STDEV).

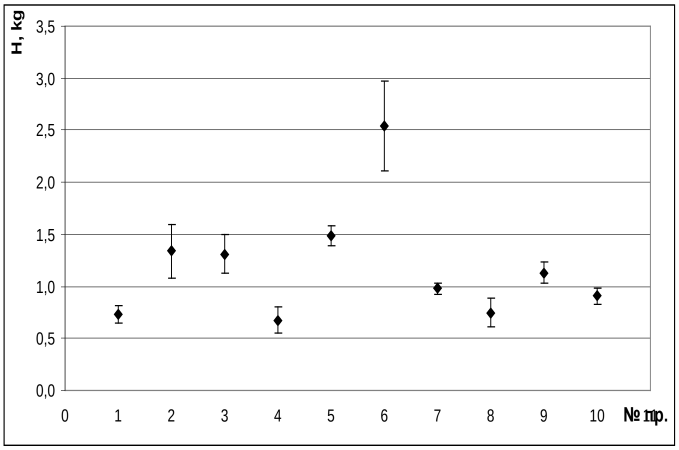

Fig. 6. Bread formulations crumb firmness

The results were processed and an equation for the firmness of the crumb of the component mixture has been obtained (Equation 4). From the adequacy test, the model provided a good fit with adjusted R^2 of 0.89. Figure 7 shows the reflecting surface of the firmness of the crumb for the component mixture.

$$H = 0.78X_1 + 1.34X_2 + 1.18X_3 - 1.34X_1X_2 + 1.71X_1X_3 + 4.61X_2X_3 - 24.13X_1X_2X_3 \quad (4)$$

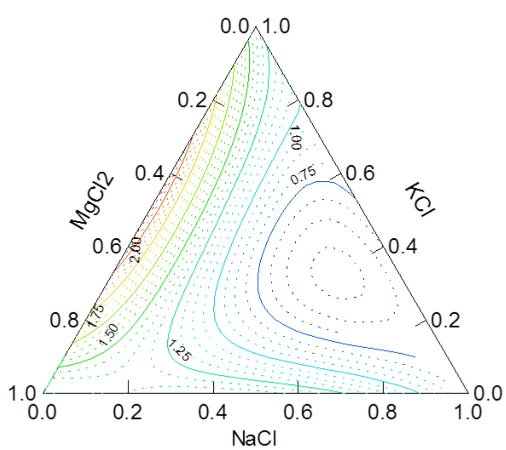

Fig. 7. Reflection surface of the firmness of the crumb of the component mixture

The hardest structure of the bread crumb is observed on the KCl-$MgCl_2$ axis, and especially at the middle where NaCl is absent or its content is negligible, which is also visible on the Figure 6 plot (sample 6). The lowest hardness values were observed in samples with higher NaCl content (samples 1 and 8) and also when proportions of NaCl-KCl are equal (sample 4). The hardness of the samples increases from the outside towards the breads center with a tendency of the NaCl content decrease (Figure 7).

After baking laboratory test of floor bread was made, an in-plant sensory test was performed to formulate initially prototypes with low sodium content. Each of the evaluators (n = 15) assessed 10 samples of bread enriched with inulin, without or with NaCl completely or partially replaced by selected salts in various concentrations. Participants familiar with the methodology of the study rated on “overall acceptance” (SV) using a 9-point hedonic scale (dislike extremely – like extremely). Table 6 shows the mean hedonic values of the bread sample treatments.

Table 6. Mean values of the sensory evaluation by sensory attribute "overall acceptance"

Sample	Overall acceptance	Sample	Overall acceptance
1.	7.8a	6.	5.7c
2.	7.7ab	7.	7.5ab
3.	6.2c	8.	8.0a
4.	7.7ab	9.	8.3a
5.	7.5ab	10.	7.8a

The ratio between the selected salts is shown in table. 3. Mean values denoted by the same letter are not statistically significant ($P < 0.05$).

After processing the data an equation for the sensory evaluation by sensory attribute "overall acceptance" was obtained (Equation 5). From the adequacy test, the model provided a good fit with adjusted R^2 of 0.76. Figure 8 shows the reflecting surface of the sensory attribute "overall acceptance" for the component mixture.

$$SV = 7.69X_1 + 7.85X_2 + 6.36X_3 - 0.13X_1X_2 + 2.11X_1X_3 - 4.38X_2X_3 + 28.8X_1X_2X_3 \quad (5)$$

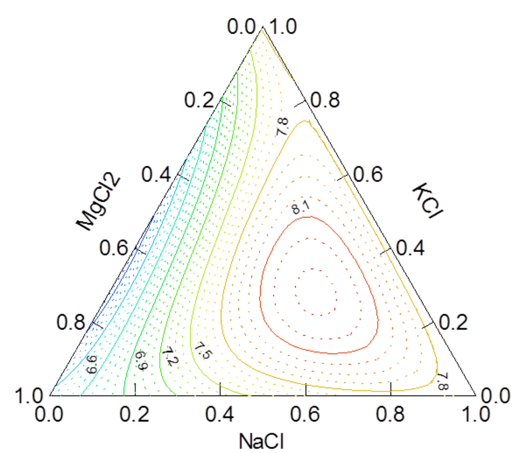

Fig. 8. Reflection surface of the sensory attribute "overall acceptance" of the component mixture

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Overall acceptance ratings (Table 6) vary from 5.7 "neither like nor dislike" (sample 6 with added KCl and $MgCl_2$) to 8.0 and 8.3 "like it very much" (sample 8 with a mixture of the three salts with predominant NaCl and sample 9 with a mixture of the three salts in predominant KCl). Figure 8 shows the highest values for "overall acceptance" in the center of the area to the NaCl peak, and the lowest towards the KCl-$MgCl_2$ axis.

For optimization of the low-salt bread formulation the following limited conditions were accepted: specific volume > 5.0, H/D index > 0.56, firmness < 1.5, and overall acceptance > 7.2. Optimization has been carried out by the superposition of the contour plots for predicted specific volume, H/D index, firmness, and overall acceptance of the formulations with low-salt content. The optimum area for the ingredients in the mixture for the formulations is presented on Figure 9 (the darkened area).

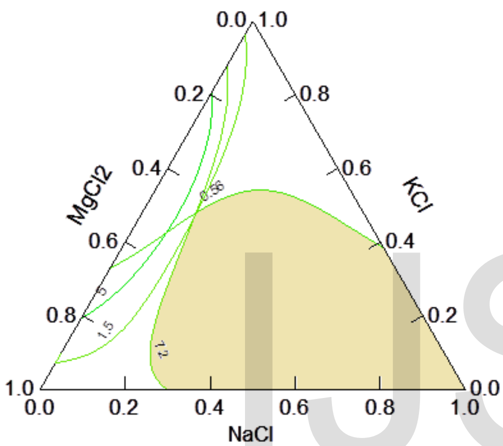

Fig. 9. Graphic optimization of the three component mixture

The quality of the inulin enriched bread was lower than that of the control. Results of samples B&C (2 to 4% added Fibrulin instant) were the closest to the control in terms of the specific volume, index H/D and consumer acceptance. The consumer acceptance test showed all the sensory attributes rated the highest (x≥7) for bread with 4% supplement.

The results related to the salt reduction demonstrated the lowest specific volume in samples with absence of NaCl.

The lowest H/D index was observed around the KCl peak, e.g. with an increase in the concentration of this salt in the mixtures the values of this parameter decreased. The firmness of the samples increased from the outside to the center with the tendency of the NaCl content to decrease.

The sample with the mixture of the three salts in predominant NaCl (sample 8) and the sample with the mixture of the three salts in predominant KCl (sample 9) received the highest ratings from the consumers.

The patterned zone on the Figure 9 shows the optimum area of the studed three components mixture of salts.

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