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What was the optimum formulation of a healthful bread with less added NaCl and more dietary fiber using surface response methodology?

Watson Dana Z*, Hernández María, Colombo María Elena, Lema Silvia N and Vázquez Marisa B*

Universidad de Buenos Aires, Escuela de Nutrición, Facultad de Medicina, Argentina

Abstract

There is an association between high NaCl intake and high blood pressure. The World Health Organization recommends salt intakes up to 5 g/day (NaCl) and a total fiber intake of 25 g/day. In Argentina it is estimated that 190 g of bread per capita are consumed per day, with an average salt content of 2%, providing approximately 4 g of NaCl to the daily intake. The objective was optimizing the formulation of a bread using the response surface methodology (RSM) in blind acceptability and with nutritional information. A Box-Behnken design was applied to the formulation prototypes for 3 critical factors: added NaCl (levels: <65%, <50% y <35%), dietary fiber (levels: >15%, >50% y>75%) y yeast (levels: <50%, conventional y>50%), taking as reference the white bread. The 15 prototypes were evaluated by 45 consumers in blind acceptability and with nutritional information. The same consumers participated in both stages. A 10-point scale was used (1=dislike extremely and 10=like extremely). The determination of the optimal bread/s was obtained via RSM. The sample of consumers consisted of 53.3% men with a median age of 28 years. The interaction stage*added NaCl was significant as the highest average acceptability values were for prototypes with <35% NaCl added for any of the stages. The added NaCl*dietary fiber interaction was also significant as the highest average values of acceptability were for prototypes with <35% added NaCl for any of the dietary fiber levels. By using the RSM it was possible to predict the optimal level of added NaCl, dietary fiber and yeast, for an acceptability of 7 points, for the blind stage and with nutritional information. The optimal formulation of a healthy bread obtained was <35% of added NaCl>75% of dietary fiber and >50% yeast in relation to white bread.

Introduction

The World Health Organization (WHO) recommends the intake of salt amounting up to 5 g per day (NaCl) and a total fiber intake of 25 g/day, from different sources, such as vegetables, fruits, grains and whole grains [1,2].

There is an association between high NaCl intake and high blood pressure (HBP), which is the most important risk factor in the development of cardiovascular diseases and strokes [1,3]. It has been observed that modest reductions of NaCl in the diet contribute substantially to reduce cardiovascular events in the population [4]. In Argentina and around the world, actions are being taken to reduce the intake of NaCl [5].

There formulation of processed products is one of the most cost-effective strategies to achieve a decrease in NaCl intake. In Argentina it is estimated that 190 g of bread per capita is consumed per day, with an average salt content of 2%, providing approximately 4 g of NaCl to the daily intake [6-8].

Diets with high dietary fiber content have proved to have positive effects on satiety, intestinal peristalsis, reduction of cholesterol values and improving glycemic control [9]. According to the Argentina's National Survey of Nutrition and Health (ENNyS), the median daily fiber intake is 9.39 g. 97.2% of women between 10 and 49 years old have inadequate fiber intake [10].

During the development or reformulation of a product, both analytical and affective sensory tests are needed to evaluate acceptability [11]. Response surface methodology (RSM) is one of the methods used to optimize products and processes, since it allows reducing the number of tests that are carried out and choose the best alternative within a series of possible options [11,12].

It is important to keep in mind that nutritional information accompanying a product plays a fundamental role in the consumer's response, influencing expectations and perceptions referred to the product, as well as its acceptability [13].

Based on what has been mentioned above, the development of bakery products with low NaCl content and high fiber content is a strategy in orderto offer consumers food choices aimed at healthy eating.

The objective of the present work was to optimize the formulation of a bakery product (bread) using the RSM in 2 stages (blind acceptability and with nutritional information).

Materials and methods

The work protocol was approved by the Comité de Ética Humana, Secretaría de Ciencia y Técnica, Facultad de Medicina, Universidad de

***Correspondence to:** Dana Zoe Watson, Universidad de Buenos Aires, Escuela de Nutrición, Facultad de Medicina, Argentina, E-mail: dwatson@fmed.uba.ar

Marisa Beatriz Vázquez, Universidad de Buenos Aires, Escuela de Nutrición, Facultad de Medicina, Argentina, E-mail: mbvazquez@fmed.uba.ar

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Buenos Aires. Consumers were invited to participate in the study and the dynamics of the test were explained to them. The participation was voluntary, and the informed consent process was previously carried out.

According to the recommendations for a healthy diet it is necessary to reduce the consumption of NaCl and increase fiber consumption. When including these recommendations in the formulation of healthy bread, it was necessary to consider the effect of the yeast facing these modifications in the final texture of the product. So, the critical factors were:

- Added salt (added NaCl)
- Dietary fiber
- Fresh yeast

A Box-Behnken design was applied to the formulation prototypes because it has a lower number of experimental units compared to the 3n designs, as it is formed with the combination of 2n designs and incomplete block designs. The critical factors were given determined three levels each, taking as a reference the white bread (Table 1).

Table 2 shows the complete set of combinations of treatments for a factorial of 2n for each pair of factors accompanied by level 0 of the remaining factors and includes 3 replicas of the design center [14], resulting in 3 identical prototypes (prototypes G, H and I) with the central levels for each factor and another 12 different prototypes making it a total of 15 prototypes.

The percentages of decrease in the content of added NaCl and increase in dietary fiber were indicated taking into account that these modifications can have a positive impact on the prevention of chronic non-communicable diseases (CNCD) in the consumers of healthy bread. The yeast levels were based on taking into account possible changes in the texture of the bread, due to the effects of variations in the content of added NaCl and dietary fiber on this sensory attribute.

Development of bread formulations

For the formulations of the bread the following materials were employed: water, wheat flour 000, whole-wheat flour, fresh yeast, salt (NaCl), sucrose and sunflower oil. The sequence of operations for the preparation was: mixture of ingredients (1/4 part of wheat flour 000 and whole-wheat flour, yeast, sugar, water and sunflower oil), fermentation, incorporation of the rest of ingredients, kneading, proofing, stretching of the dough and formation of the breads, leavened, cooking in convector oven and cooled. The formulation of each prototype, in percentages of ingredients, are presented in Table 3.

Prototypes' compositions were obtained theoretically. In order to verify it, the analytical determinations of the prototype "O" were carried out for humidity [15], ashes [16], carbohydrates (calculated by difference as 100-[%H+%C+%G+%P+%F]), proteins (N×6.25) [17], fat [18], total dietary fiber [19] and sodium (previous treatment AOAC 968.08–2000 [20] and later quantitative analysis by atomic absorption spectrometry) (Table 4).

For this formulation, analytical values coincided with the one obtained theoretically, thus the theoretical composition was considered adequate for the other formulations.

Table 1. Coding of factors

Factor	Added NaCl	Dietary Fiber	Yeast
-1	<65%	>15%	<50%
0	<50%	>50%	Conventional
+1	<35%	>75%	>50%

Sensory analysis of blind acceptability and with nutritional information

The 15 prototypes were evaluated by a panel of 45 habitual consumers of bread [21].

The prototypes were evaluated in 2 stages: blind acceptability and acceptability with nutritional information. Consumers were the same in both stages. The time of the interval between the first and the second stage was 1 week [22]. In each stage, the 15 prototypes were evaluated in 2 sessions: session 1=evaluation of 8 prototypes and session 2=evaluation of 7 prototypes (interval between sessions of 30 minutes).

- STAGE 1: Blind acceptability: the samples were presented monadicallyin balanced order, following a Latin Squares design. Each

Table 2. Experimental design of the samples

Prototype	Added NaCl	Dietary Fiber	Yeast
A	<35%	>15%	Conventional
B	<35%	>50%	<50%
C	<35%	>50%	>50%
D	<35%	>75%	Conventional
E	<50%	>15%	<50%
F	<50%	>15%	>50%
G	<50%	>50%	Conventional
H	<50%	>50%	Conventional
I	<50%	>50%	Conventional
J	<50%	>75%	<50%
K	<50%	>75%	>50%
L	<65%	>15%	Conventional
M	<65%	>50%	<50%
N	<65%	>50%	>50%
O	<65%	>75%	Conventional

Table 3. Formulation of the 15 bread prototypes in percent of ingredients

Prototype	Wheat flour %	Whole-Wheat Flour %	NaCl %	Yeast %	Sucrose %	Water %	Oil %
A	52.3	5.8	0.6	2.9	1.1	32.0	5.3
B	41.3	17.7	0.6	1.5	1.1	32.5	5.3
C	40.1	17.2	0.6	4.3	1.1	31.5	5.2
D	29.1	29.1	0.6	2.9	1.1	32.0	5.3
E	53.2	5.9	0.4	1.5	1.2	32.5	5.4
F	51.7	5.7	0.4	4.3	1.1	31.6	5.2
G	40.8	17.5	0.4	2.9	1.1	32.0	5.3
H	40.8	17.5	0.4	2.9	1.1	32.0	5.3
I	40.8	17.5	0.4	2.9	1.1	32.0	5.3
J	29.5	29.5	0.4	1.5	1.2	32.5	5.4
K	28.7	28.7	0.4	4.3	1.1	31.6	5.2
L	52.2	5.8	0.3	2.9	1.1	32.1	5.3
M	41.4	17.8	0.3	1.5	1.2	32.5	5.3
N	40.2	17.2	0.3	4.3	1.1	31.6	5.3
O	29.2	29.2	0.3	2.9	1.1	32.1	5.3

Table 4. Analytical determinations of the prototype "O" in 100 g of product

Determination	Result
Energy	305 kcal
Humidity	29.1 g
Ashes	0.7 g
Carbohydrates	48.8 g
Proteins	8.4 g
Fat	8.4 g
Dietary Fiber	4.4 g
Sodium	155 mg

sample of 1 slice of bread of 10±1 gram, was served in transparent disposable tray (7×7×4 cm) and coded with a 3-digit number. Consumers tested the samples, waiting 1 minute between each one and wiping their mouths with water. The global acceptability was evaluated using a 10-point scale (1=dislike extremely and 10=like extremely) in a self-administered form.

- STAGE 2: Acceptability with nutritional information: The nutritional information included: the name of the product (BREAD), the percentages of decrease in NaCl added, the percentages of increase in dietary fiber and the ingredients of each prototype. The dynamics of the test and the scale used was equal to STAGE 1, only consumers were asked to read the nutritional information before testing the samples.

At the end, the participants completed the socio-demographic questionnaire (self-administered) that included the following variables:

- Age: measured in years
- Gender: categorized Female/Male
- Work: categorized in Yes/No
- Study: categorized in Yes/No
- Type of bread consumed: categorized in White bread/Whole-wheat bread/Both
- Moment of the day in which it consumes bread: categorized in Breakfast/Lunch/Tea time/Dinner/Between meals/Others
- Type of bread you usually buy: categorized in Craft based bread/ Industrial bread/Both

Statistical analysis

The sample was described according to the characterization variables. The numerical variable (age) was expressed as median and interquartile range. The dichotomous and categorical variables were expressed in absolute values and percentages.

Analysis of the Variance (ANOVA) was performed on acceptability scores of the 15 prototypes. Averages were compared using Fisher's LSD method. A value of 5% was considered significant for the comparison of averages.

The Genstat software was used (Version 19, VSN International, Hemel Hempstead, United Kingdom).

The determination of the optimal bread/s was obtained via RSM with the MINITAB software (Version 15, Minitab Inc.). A significance level of 5% was considered in the analysis.

Results

The 15 prototypes of the bakery were elaborated successfully by the baker cook, according to a standardized recipe.

The sample of consumers consisted of 53.3% of men, with a median age of 28 years. 57.8% and 71.1% indicated that they study and work respectively. When asked about the type of bread they consumed 40% of the evaluators reported only white bread, while 33.3% indicated consuming white and whole-wheat breads. As for the bread they bought 51.2% referred to buying only industrial bread and 24.4% bought both: industrial and craft-based bread. Breakfast and tea time were the moments most chosen by consumers to eat bread (33) (Table 5).

Analysis of the blind acceptability and with nutritional information of the prototypes

Table 5. Characterization of the 45 consumers

Characteristics	Consumers n (%)
Age (years) - Median (IR*)	28 (11)
Gender	
Female	21 (46.7%)
Male	24 (53.3%)
Study	
Yes	26 (57.8%)
No	19 (42.2%)
Work	
Yes	32 (71.1%)
No	13 (28.9%)
Type of bread consumed	
White bread	18 (40.0%)
Whole bread	12 (26.7%)
Both	15 (33.3%)
Type of bread you usually buy	
Craft based bread	11 (24.4%)
Industrial bread	23 (51.2%)
Both	11 (24.4%)
Day time	
Breakfast	32 (32.3%)
Lunch	10 (10.1%)
Tea	33 (33.3%)
Dinner	11 (11.1%)
Between meals	11 (11.1%)
Others	2 (2.1%)

*IR: Interquartile range

Of the total prototypes evaluated, the one that obtained the highest average score of acceptability by consumers was prototype C, in both blind stage (7.02, DS: 1.79) and with nutritional information (6.98; DS: 1.50). The following prototypes were also found with average scores of acceptability above 6.5: A, J, K and D.

The prototypes with 65% reduction of added NaCl presented acceptability values of 6 or less points, both blindly and with nutritional information (except the prototype O in blind stage).

Subsequently, factorial ANOVA of the acceptability of the 15 prototypes was performed.

The interaction stage*added NaCl was significant for a p value=0.012. It is important to remember that, when using the Box-Behnken design to obtain the prototypes, 3 replicas of the design center are included, resulting in 3 prototypes equal with the central levels for each factor and another 12 different prototypes, making a total of 15 prototypes. Due to this, different repetitions are observed in the NaCl factor levels added for each stage. The highest average acceptability values were for the prototypes with <35% NaCl added for any of the two stages (Table 6).

The added NaCl*Dietary fiber interaction was also significant (p=0.048). As mentioned above, the number of repetitions varies, depending on the levels of added NaCl and dietary fiber that interact, due to the Box-Behnken design used. The highest average values of acceptability were for the prototypes with <35% added NaCl for any of the dietary fiber levels and no difference in acceptability was observed. The lowest average values were for bread prototypes with <65% of added NaCl. But when the reduction of NaCl was <50% and the amount of added fiber of 75% the acceptability was significantly higher than for the bread with 15% added fiber (Table 7).

No statistically significant differences were found for the interactions Stage*Dietary fiber (p=0.594), Stage*Yeast (p=0.439), added NaCl*Yeast (p=0.334) and Dietary fiber*Yeast (p=0.939).

Determination of the optimum bread/s

For the implementation of the graphics by the RSM it was necessary to work with an ordinal numerical scale, so the levels of each factor were expressed in grams, since the "Conventional Yeast" level was not a numerical value.

The response surface graphs explain the effect of the independent variables (added NaCl, Dietaryfiber and Yeast) on the dependent (Acceptability).

The results of blind acceptability and with nutritional information stages are presented in Figure 1.

When acceptability was carried out blindly, the following was observed:

A) Acceptability graph in function of added NaCl factor and dietary fiber factor: it can be seen that the acceptability of the bread is increasing as the amount of added NaCl and dietary fiber increases.

B) Acceptability graph in function of added NaCl factor and Yeast factor: it is observed that the higher the content of added NaCl and Yeast, the greater the acceptability of the product.

C) Acceptability graph as a function of the Dietary fiber factor and the Yeast factor: the acceptability of the Dietary fiber goes down to> 50% and then rises to reach the maximum acceptability value with the highest level of Dietary fiber. Regarding the amount of Yeast, the highest values of acceptability are observed when the concentrations are at extreme levels.

In the stage with nutritional information, the results were:

D) Acceptability graph in function of added NaCl factor and Dietary fiberfactor: the acceptability of the bread is increasing as the amount of added NaCl increases. The same happens with the content of Dietary fiber.

E) Acceptability graph in function of added NaCl factor and Yeast factor: it is seen that the higher the content of added NaCl and of Yeast, the greater the acceptability of the product.

F) Acceptability graph according to the Dietary fiber factor and Yeast factor: acceptability goes down to> 50% and, then, rises until reaching the maximum acceptability value with the highest level of Dietary fiber. Regarding the amount of Yeast, the highest values of acceptability are observed when the concentrations are at extreme levels.

Table 6. Acceptance averages for the Stage×NaCl added interaction

		NaCl added			LSD*	LSD**
		<65%	<50%	<35%		
		Acceptability (repetitions)	Acceptability (repetitions)	Acceptability (repetitions)		
Stages	Blind Acceptability	6.01 (180)	6.27 (315)	6.69 (180)	0.22	0.19
	Acceptability with Nutritional information	5.48 (180)	6.15 (315)	6.82 (180)	0.22	0.19

*Least Significant Difference ($p<0.05$) to compare means with the same number of repetitions
**Least Significant Difference ($p<0.05$) to compare means with different number of repetitions

Table 7. Acceptance averages for the NaCl added×dietary fiber interaction

		Dietary Fiber			LSD*	LSD**
		>15%	>50%	>75%		
		Acceptability (repetitions)	Acceptability (repetitions)	Acceptability (repetitions)		
NaCl added	<65%	5.72 (90)	5.65 (180)	5.92 (90)	0.44	0.36
	<50%	5.97 (180)	6.16 (270)	6.57 (180)	0.44	0.36
	<35%	6.90 (90)	6.72 (180)	6.66 (90)	0.44	0.36

*Least Significant Difference ($p<0.05$) to compare means with the same number of repetitions
**Least Significant Difference ($p<0.05$) to compare means with different number of repetitions

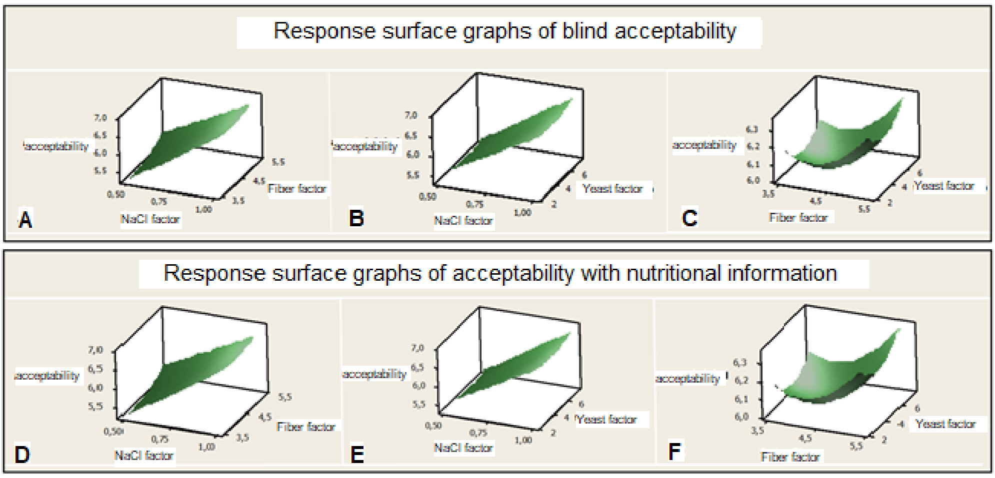

Figure 1. Response surface graphs of acceptability: Blind and with nutritional information

By using the MSR it was possible to predict the optimal level of added NaCl, Dietary fiber and Yeast, for an acceptability of 7 points, both for the blind stage and with nutritional information. In this way, an optimal prototype was obtained for each stage (Table 8).

Taking into account the nutritional goals for a healthy diet and recommendations for the daily intake of dietary fiber [2] and that both prototypes have the same levels of added NaCl and Yeast, but not of Dietary fiber, it was decided to define as the optimal prototype the bread with: <35% of added NaCl; >75% of dietary fiber; >50% yeast than white bread.

Discussion

The use of the Box-Behnken design allowed us to formulate and elaborate a smaller number of experimental units to optimize the healthful bread, as 15 prototypes were made, instead of 27. Other researchers have used it for the optimization of the ingredients in the development of products with good results. Some works include foods such as noodles with different combinations of wheat flour, soybean and cassava starch [23]; wheat and soy crackers with added turmeric [24]; and yogurt [25].

The 15 prototypes of the bread were elaborated successfully. In Ireland, Lynch EJ *et al.* [26] studied the impact of NaCl reduction on the characteristics of dough and bread, finding that it can be reduced from 1.2% to 0.3-0.6% without meaningful affecting them.

According to Carrillo E *et al.* [13], the nutritional information that accompanies a product plays a fundamental role in the consumer's response, asit can influence their expectations and their perceptions of it, as well as its acceptability. NaCl reduction content in food products usually generates a negative impact on the expectation and/or the acceptability of these [22,27]. In contrast, in the incorporation of fiber or substitution of sugar by other sweeteners can have a positive impact [28,29].

When performing the acceptability test in the blind stage and, 1 week later, with nutritional information, differences were found for NaCl levels. When the reduction was <65% in the stage with nutritional information, consumers gave a lower score. For the reduction levels of <35% and <50% NaCl there were no differences. When the reduction of NaCl was <50% and the amount of added fiber of 75% the acceptability was significantly higher than for the bread with 15% added fiber. This could be due to the interaction of the ingredients in relation to the flavor in the bread favoring the acceptability [30,31].

Some strategies directed at reducing the sodium content in foods, without affecting their acceptability, consist of progressive reductions in the amount of NaCl. Girgis S *et al.* [32] achieved an acceptability rate of 25% with the reduction of the salt content in white bread in 6 weeks. In contrast, Antúnez *et al.* [33] studied the partial replacement of NaCl with KCl (30% reduction of the NaCl content in bread with a KCl replacement). Other authors achieved good levels of acceptability on the part of consumers with decreases in the amount of NaCl added between 20 and 30% [6,34].

Table 8. Optimal prototypes of blind acceptability and with nutritional information

Factor	Blind Acceptability (acceptability=7)	Acceptability with Nutritional information (acceptability=6.999)
NaCl added	<35%	<35%
Dietary Fiber	>15,43%	>75%
Yeast	>50%	>50%

The RSM was used to achieve the optimization of the bread and to predict the optimal levels for each factor, for an acceptability of 7 points. The effectiveness of RSM in the development and optimization of cereal-based products was also highlighted by other research groups [24,35,36].

As a result of the optimization, two optimal formulations of bread were obtained where the difference was in the fiber content: when the prototypes were evaluated blindly, the optimal level was >15% fiber, but when they received the nutritional information, the optimal level of fiber increase was >75%. One might think that consumers have knowledge about the health benefits of fiber intake and this influenced acceptability [37]. A study carried out by Królak *et al.* [38] indicated that those consumers who have knowledge about nutrition attach more importance to fiber intake, to the nutritional information of the products and also more frequently consume whole or fiber enriched breads. So, optimal healthy bread contains 35% less added NaCl, 50% more yeast and 75% more fiber than white bread. The nutritional quality of the bread was evaluated through the application of the Nutrient Profile Model proposed by the Pan American Health Organization (PAHO) [39] and it was observed that it presented the critical nutrients (free sugars, fats saturated, trans fats, total fats, sodium and other sweeteners) aligned to the limits proposed by profiling.

Conclusion

The optimal formulation of healthy bread obtained was <35% of added NaCl>75% of dietary fiber and >0% yeast in relation to white bread.

A product with these characteristics would be a very good strategy to offer different food options to the consumer that aim at a healthy diet. That is why the next step of research will be the measurement of its acceptability in a large number of consumers.

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Competing interest

The authors have not financial or non-financial, professional, or personal competing interests for consideration during the review process.

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