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Production and Sensory Evaluation of Novel Cheeses Made with Prebiotic Substances: Inulin and Oligofructose

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Abstract

In recent years, the processing and consumption of functional foods worldwide have greatly increased. These foods benefit the body functions which improve consumers' health and also reduce the risk factors that cause the onset of disease. Furthermore, prebiotic substances favor the multiplication of beneficial intestinal bacteria rather than harmful ones. The purpose of this study was to conduct the sensory evaluation of two functional cheeses containing inulin and oligofructose as a distinctive ingredient, including testing a cheese made with conventional ingredients, called control cheese. Affective type tests, which measured the degree of liking or disliking, were conducted using a verbal 7-point hedonic scale. According to the inclusion and exclusion criteria, 57 untrained judges were selected. This study is a quantitative, analytic and experimental-cross design. Statistical analysis of the data was performed by ANOVA with repeated measures. The results show a similar average degree of liking for the three cheeses, above 5 on the scale or "like". By analyzing the critical level and the result of the Mauchly's sphericity test, it is concluded that there is no statistically significant difference in the degree of liking for the three cheeses. Therefore, the addition of prebiotics to artisanal cheeses achieves to satisfy consumers and provide them benefits superior to those provided by traditional foods.

Keywords

Cheese, Inulin, Oligofructose, Functional Foods

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1. Introduction

Lately functional foods have caught the attention of the population, becoming more important and encouraging development, and consequently, the evolution of many food products.

The interest of this study was to perform sensory evaluation of functional cheeses including prebiotics as ingredients. Specifically, the purpose was to develop three types of cheeses, two of which included in its composition inulin and oligofructose respectively and one with conventional ingredients (control cheese), and to determine the degree of liking of such products by untrained judges.

Generally, functional food referred to any product, food or food ingredient that might provide consumers with higher benefits than those offered by traditional foods. The "functional" term implies some identified value to improve health, including the reduction of disease risk to the persons who consume it [1] [2].

Currently, there are different kinds of products commercially available with prebiotic substances that give food aforementioned characteristics [3]. The "prebiotics" are non-digestible food ingredients that have beneficial effects on the host by selectively stimulating the growth and/or activity of one type or a limited number of bacteria in the colon [4] [5]. They are part of which is known as functional fiber. This group of substances can cause a large number of positive physiological effects, through a more efficient digestion, increasing the absorption of calcium and other minerals increasing fecal weight, shortening the gastrointestinal transit time and reducing blood lipid levels [2] [4] [6].

Also, it has been found that incorporations of prebiotics to different foodstuffs favor the water absorption, prevent intestinal infections, promote the inhibition of pathogens such as *Escherichia coli* and *Clostridium* spp., and reduce risk of colorectal cancer [7].

Since fructans are currently recognized as substances with prebiotic activity, they are used in this investigation as ingredients for obtaining artisanal functional cheeses. Fructans are storage carbohydrates (non-structural carbohydrates) presented in many plants, fruits and grains and thus form part of our daily diet. They can be used by industry as foods ingredients, offering significant technological advantages. The most prominent ingredients are inulin and oligofructose; characterized by their links β-(2-1) between the fructose units with a polymerization degree varying between 2 and 60 units [5]-[9].

Inulin has a neutral soft flavor and is moderately water soluble. It can be used as a sugar substitute, fat substitute, texture modifying agent and/or foam and emulsions stabilizer. Inulin can be incorporated into dairy products, fermented products, jellies, airy desserts, mousses, ice cream and bakery products. Oligofructose is more soluble than inulin and moderately sweet. In the industry, it can be used in reduced calorie foods such as yogurt with fruits, fermented milks, ice cream and drinks. The inulin and oligofructose improve the texture of final product [2] [3] [5] [6] [10].

One of the most important areas within the world of functional foods is related to dairy products. Within them, cheese can be a clear and not entirely depleted source of functional food. Their nutritional data may vary, but in general, are rich in protein, lipids, minerals (calcium, phosphorus, sodium, etc.) and vitamins. Cheeses were selected, in this paper, as a vehicle for the incorporation of inulin and oligofructose, considering its nutritional characteristics, and the possibility of adding, during its production process, new ingredients and/or changing its composition.

When foods are designed or when changes are made to existing foods, it is of great interest to carry out a sensory evaluation as quality control, besides physical, chemical and microbiological tests. For this research, affective test was chosen, as it was adequate to know the judges' subjective reactions towards the products, measuring the degree of liking or disliking [11].

Finally, developing artisanal cheeses was decided on the basis that it was an ancient tradition in our country and because the appreciable production volume due to the high demand of the local market.

2. Experimental Section

2.1. Population

This is a quantitative, analytical and experimental-cross designed study. 57 untrained judges of both sexes and from 18 to 65 years old were selected. They were chosen on the basis of well-defined selection criteria described below.

Selection Criteria

Inclusion criteria: people of both sexes, 18 - 65 years old, interested in participating in the research, who were

regular consumers of artisanal cheeses, with availability to attend the session when prompted.

Exclusion criteria: allergy sufferers, people with colds, stomach disorders, toothache, pregnant women, people involved in the investigation or having knowledge thereof, people who work with food as researchers or employees of processing food factories, and people who periodically perform sensory evaluations.

Moreover, the judges were asked to avoid being tired, not using perfumes or scented soaps, not to smoke, not to drink alcohol, not eat food with very invasive flavors, no coffee, at least one hour before attending the sensory evaluation session. This request was made in order to reduce to a minimum the existence of factors that influence the results.

2.2. General Methods

For the preparation of the three types of cheeses, the methodology proposed by the National Institute of Industrial Technology and Spreer was taken as reference [12] [13]. Fresh white milk, with characteristic odor and slightly sweet flavor was used. The pH value of milk was determined by using test strips (DF Universal Test Paper).

Precipitation of milk proteins was carried out by enzymatic coagulation. Rennet (Renei-Tres Coronas S.A., Buenos Aires city, Argentina) was used in the amounts recommended by the supplier (300 g/1000 L). Inulin (Orfati®GR) and oligofructose (Orafti®P95) were added according to the specifications present in the article 1385 and 1386 of the Argentine Food Code [14] Detergent suitable for contact with food, potable water and sodium hypochlorite solution (0.1% - 0.5% v/v) were used for cleaning and disinfection of utensils.

2.3. Preparation of the Cheeses

2.3.1. Control Cheese

The control cheese (CC) was prepared using the strategy described below. Initially, 5 liters of raw milk (pH 6) were filtered using a strainer and linen. The milk was pasteurized (68˚C ± 0.5˚C, 15 min) and cooled down to 36˚C ± 0.5˚C. Then, 12 g of commercially available yogurt and 10 g of calcium chloride was added. The mixture was gently stirred during 5 minutes and then it was kept at rest for 30 minutes. 1.5 g of rennet was added to precipitate milk proteins. After coagulation time of approximately 1 hour, the curd was cut into 8 cm^3 cubes and it was gently stirred during 10 minutes raising the temperature to 40˚C, then the whey was mostly drained. The curd was transferred to 1 Kg perforated round mould and kept at room temperature for 1 hour. Afterwards the mould was turned and left to stand for 30 min. Once the curd had drained, the cheese was withdrawn from mould and salting was carried out by direct contact with table salt for 1 hour. Then the cheese was washed with potable water, weighed, wrapped in polyethylene film and held in a refrigerator at 4˚C ± 1˚C to promote the maturation processes for a period of 20 days.

2.3.2. Cheeses with Inulin and Oligofructose

For the incorporation of inulin and oligofructose on the different types of cheeses (IC and OC respectively) the same methodology as described for the production of (CC) was used. In each case, 5 liters of pasteurized milk (68˚C ± 0.5˚C, 15 min), 12 g of commercially available yogurt and 10 g of calcium chloride were incorporated. The inclusion of 15 g of each prebiotic substance was performed before the addition of 1.5 g of rennet. As explained in section 2.2, the dose of each prebiotic substance was selected in accordance with specifications present at the Argentine Food Code [14]. The process concluded without modifications in the methodology. The Production of each type of cheese was carried out in duplicate.

In order to comply with the article 558 and 605 of the Argentine Food Code, both pasteurized milk used for the production of the three types of cheeses as samples of these products (CC, IC and OC) received the relevant microbiological analysis [15]. The tests for pasteurized milk included, plate count of aerobic bacteria 37˚C (ISO 4833:2003), coliform bacteria 30˚C/g (ISO 4832:2006), *Escherichia coli*/mL (ICMSF Method 1), *Staphylococcus aureus* coagulase positive/g (ISO 6888-1:1999) and *Salmonella* spp./25 g (BAM-FDA: 2007). In the case of the samples of CC, IC and OC, tests used were plate count of aerobic bacteria 37˚C (ISO 4833:2003), coliform bacteria 30˚C—Most Probable Number (ISO 4831:2006), coliform bacteria 45˚C—Most Probable Number (ICMSF Method 1), *Staphylococcus aureus* coagulase positive/g (ISO 6888-1:1999), *Salmonella* spp./25 g (BAM-FDA: 2007), yeast and mould count (ISO 6611-IDF 94: 2004).

2.4. Sensory Evaluation of Produced Cheeses

After controlling the physico-chemical and microbiological quality of produced cheeses, sensory evaluation tests were conducted. It was decided to perform affective sensory tests, also called hedonic tests, that attempt to quantify the degree of liking or disliking of a product [11] [16]-[19]. For this, as mentioned above, 57 untrained judges were selected. They were chosen carefully to insure that the results will generalize to the population of interest.

It was used a 7-point verbal hedonic scale with three positive categories in the upper pole, a centered neutral category and three negative categories in the lower pole. Each category represented psychologically equal steps or changes in hedonic tone [11] [16] [17] (**Table 1**).

The preparation and serving of samples were made under controlled conditions, so that biasing factors were minimized [11] [20] [21].

The testing area was kept quiet and it was climate controlled to facilitate judges' concentration. Also, the area was well lit with balanced daylight-type fluorescent bulbs to prevent differences in the color of the samples.

Before the judges start the testing were given instructions on how to perform the sensory evaluation, both verbally and in written form on the score sheet.

Booths were not used, instead judges were seated at small tables, which were arranged in such a way that they do not face each other. The advantage of this situation is that the testing area can be set up fairly quickly and the whole group can receive any verbal instructions simultaneously.

The tables had enough space to place a serving tray which contained a dish with each sample, a fork, a knife, a napkin, three score sheets (one for each sample), a pen to write answers and a plastic water glass. Judges were asked to drink a little amount of water between sample and sample to avoid overlapping of flavors. All the serving utensils were white colored, to prevent distortion in the perception of attributes, and were disposables, so that they could be discarded after used.

Samples were blind labeled with random four-digit codes (**Table 2**) so that judges do not make decisions based upon labels, but rather on their sensory experiences. Those codes were written on the samples containers (dishes). Furthermore, samples order was randomized to avoid bias due to order presentation [21]. So, samples were assembled for each judge on the tray in the sequence that they were to be evaluated.

The samples were served to judges at a temperature of 14˚C ± 4˚C. Each sample weighed approximately 10 grams and measured about 9 cm^3 (about 3 × 3 × 1 cm in size). The sessions were performed from 10 am to 11:30 am, and from 4 pm to 6 pm [21].

Table 1. Numerical score for categories of verbal hedonic scale used in sensory analysis tests.

Score	Categories
7	I like extremely
6	I like very much
5	I like
4	Neither like nor dislike
3	I dislike
2	I dislike very much
1	I dislike extremely

Table 2. Random codes used for each sample.

Samples	Nomenclature	Random codes
Control Cheese	CC	3749
Inulin Cheese	IC	4936
Oligofructose Cheese	OC	7463

2.5. Statistical Analysis of the Data

The data obtained for each condition (type of cheese) were analyzed by ANOVA with repeated measures that suits when comparing categorical related groups is necessary. In this case, the same subjects were measured with a hedonic scale (like/dislike) after trying three different kind of cheese. Assumptions of not significant outliers, normality, homogeneity of variances, and sphericity, were explored.

3. Results and Discussion

Conventional cheeses were developed based on the methodology of INTI—Argentina (National Institute of Industrial Technology, Argentina). Some modifications were performed in order to optimize the process. Slight changes in the amounts of some ingredients, gel formation time, coagulation time, pressing and salting technique allowed to obtain a product with the desired appearance (**Figures 1 (a)-(c)**). Using 5 liters of raw milk the weighing was 618 g. This is encouraging, since using 10 liters of milk it is expected to obtain a cheese from 1 to 1.3 kg [12].

Inulin and oligofructose incorporation during the cheese making process was performed unhindered and coagulation was carried out normally in both cases (**Figure 2**). Weighing obtained for the cheese with inulin was 747 g and 755 g for the cheese with oligofructose (both using 5 liters of fresh milk).

Developed cheeses were kept in a refrigerator for 20 days. Its outer surfaces were flat and showed no deformations. Abnormal colors, spots, erosion or cracks (usually caused by the presence of molds, yeasts and bacteria) were not observed in the crust.

The results of the sensory evaluation of the three types of cheeses show that the average degree of liking for CC, IC and OC was similar (mean and standard deviation of 5.58 ± 1.12, 5.19 ± 1.16 and 5.77 ± 0.96 respectively), each one scoring more than 5, or "Like" (**Table 3**). This means that the cheeses liked the judges whether or not containing new ingredients such as inulin or oligofructose. These results are encouraging, since it suggests that the addition of prebiotic substances would not imply a decrease in the consumption of the products. In addition, there were no significant differences in outcomes by age or sex among judges.

(a) (b) (c)

Figure 1. Development of conventional cheeses (QC) (no additional components). (a) Mass after cutting the curd into square cubes of about 8 cm^3; (b) Curd after removing of whey; (c) Cheese after pressing and salting.

(a) (b)

Figure 2. Development of cheeses which include in its composition inulin (IC) and oligofructose (OC) respectively. (a) IC vacuum packed; (b) OC vacuum packed.

Table 3. Degree of liking found for each type of developed cheese.

Type of cheese	Hedonic Scale Score*
CC	5.58 ± 1.12
IC	5.19 ± 1.16
OC	5.77 ± 0.96

*Mean ± Standard Deviation (n = 57), one-way repeated measures ANOVA.

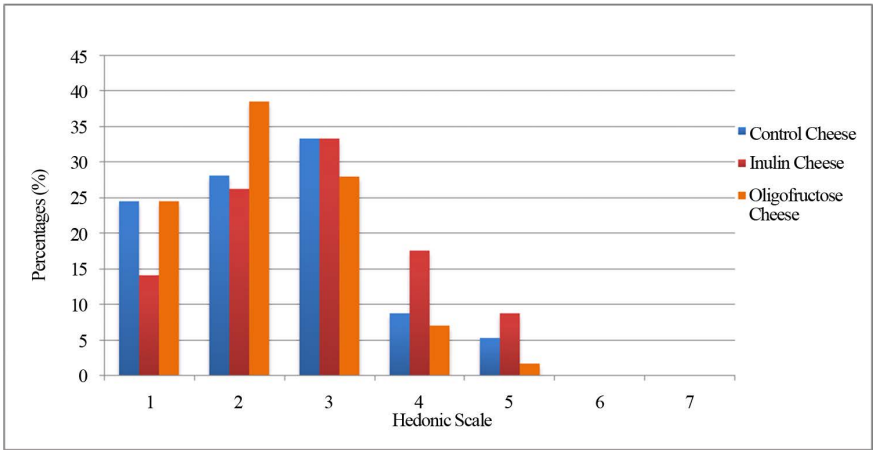

Figure 3. Degrees of liking and disliking for the different types of developed cheeses.

However, in **Table 3** and **Figure 3** it can be seen a slight tendency towards the OC cheese.

When analyzing multivariate contrasts the null hypothesis was rejected, since the critical level or significance (sig.) associated to each of the statistics (Pillai's Trace, Wilks' Lambda, Trace Hotelling and greater Root Roy) was 0.034 (less than 0.05). To test that the variances of the differences between two levels of repeated measures factor are equal, the RM procedure provides the Mauchly's sphericity test. As the critical level associated to statistical W was 0.064, the sphericity assumption was accepted.

These results show that when rejecting the null hypothesis, there would be significant differences between the mean of degrees of liking for the three types of evaluated cheeses. Specifically, observing the means, it can be seen that the degree of liking for OC was slightly higher than those found for CC and IC. The IC showed the lowest degree of liking. However, the result of Mauchly's sphericity test would indicate that the variances of the differences between two factor levels were not statistically significant, so the difference would be due to the random effect. So it could not be said that that one of these types of cheeses had a significantly higher degree of liking than the others.

4. Conclusions

The results show that the development procedure of functional cheeses is appropriate because it allows the simple, fast and economic development of the products with acceptable yields. These products provide consumers a high added value and higher benefits than traditional cheeses. Because of the easy incorporation of prebiotic substances into the production process, both inulin and oligofructose are versatile ingredients when preparing food.

After the sensory evaluation of the three types of cheeses and according to the results, it can be concluded that there is no statistically significant difference among the degrees of liking found for the three types of developed cheeses (CC, IC and OC). However, a slight trend towards the cheese with oligofructose was seen. Finally, the addition of prebiotics substances to cheeses would satisfy consumers and would provide them with health benefits.

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