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Artículos

A functional bread designed with rye flour and stevia sweetener: A sensory and microbiological annalysis

Un pan diseñado con harina de Centeno y Stevia: Un análisis sensorial y microbiológico

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Resumen:

El pan a base de harina de centeno es un producto relativamente nuevo. Este producto es un alimento funcional rico en flavonoides, mucílagos, pentosanos, vitaminas y minerales. Además, los productos a base de centeno ya han mostrado potencial para mejorar la parición de apetito posprandial, parecen disminuir la respuesta a la glucosa y la insulina y disminuyen la ingesta voluntaria de alimentos en una comida posterior. En comparación con pan blanco entero, previene la inflamación de la mucosa gastrointestinal y respiratoria, siendo una excelente opción nutricional para ser incluida en nuestra dieta diaria. El objetivo de esta investigación fue desarrollar un pan funcional a partir de harina de centeno y stevia, utilizando tres formulaciones: 50, 60 y 80% de harina de centeno. Se realizaron evaluaciones microbiológicas, fisicoquímicas y sensoriales, demostrándose que la fórmula 50/50 fue la mejor aceptada por los evaluadores voluntarios. De acuerdo con la norma NTC 1363, el producto fue clasificado como pan blando con fibra, ya que la fibra fue del 35.8% y la humedad obtenida fue de 35.65%. En el análisis microbiológico, los resultados mostraron que es un producto apto para el consumo humano y en el análisis del sabor, la textura y la presentación del pan fueron bien evaluados por los consumidores, concluyendo que este producto es adecuado como alternativa al pan de trigo en la dieta humana diaria.

Palabras clave: harina de centeno, stevia, pan funcional, fibra, calidad.

Abstract:

Rye flour bread is a relatively new development. This product is a functional food rich in flavonoids, mucilage, pentosans, vitamins and minerals. Additionally, rye-based products have previously shown to improve acute postprandial appetite ratings, appear to lower glucose and insulin response and decrease voluntary food intake at a subsequent meal. Compared to whole white bread, it prevents inflammation of both, gastrointestinal and respiratory mucosa, being an excellent nutritional option feasible to be included in our daily diet. The aim of this research was to develop a functional bread from rye flour and stevia with three formulations: 50, 60 and 80% of rye flour. The microbiological, physicochemical and sensory assessments were made, evidencing that 50/50 formula was the best accepted by the evaluators. Ac- cording to NTC 1363 norm the product was classified as a soft bread with fiber, since fiber was (35.8%) and the moisture obtained was (35.65%). In microbiological analysis, the results showed that it is a suitable product for human consumption and taste, texture and presentation analysis, this product was well-rated by the consumers, concluding that, is suitable to be merchandise as an alternative to wheat bread in daily human standard meals.

Keywords: Rye Flour, Stevia, Functional Bread, Fiber, Quality.

Introduction

Rye (Secale cereale) is a member of the grass family Gramineae, genus Secale, of which S. cereale is the most com- monly cultivated species. This grass is used extensively as a grain, cover crop and a forage crop and it is closely related to barley (genus Hordeum) and wheat (Triticum)1. The wild progenitor has not been definitely identified, although some taxonomists believe that modern rye evolved from S. montanum G., a perennial grass that grows wild in southern Europe and central Asia2. Because it is extremely winter hardy and can grow in sandy soils with low fertility, rye can be cultivated in areas that are generally not suitable for other cereals; in fact, most of the rye produced worldwide is grown in cool temperate zones, but it can also be grown in semiarid regions near deserts and at high altitudes3.

Rye grain is used in manufacturing a high variety of food products like flour, bread, beer, crisp bread, alcoholic beverages (whiskeys, beers and some vodkas), and animal fodder. It can also be eaten whole, either as boiled rye berries or by being rolled, similar to rolled oats. In this context, Secale cereale, is the second most used cereal for bread manufacture after wheat and it is also an important cereal grain for mixed animal feeds production4.

Baking quality of the rye flour often is referred as inferior compared to wheat and this is the main reason there is not bread prepared with 100% rye flour. Rye´s endosperm is strongly attached to the pericarp which makes its complete separation difficult. In consequence, rye flour always has a dark hue, which is increased as the extraction rate increases, affecting the coloring of the flour5. Thus, when the extraction is around 60% the so called "white flour" is obtained; with an 85% extraction, the flour is barely dark, and with a 90-100% of extraction a dark color and less amount of gluten. Due to a higher content of enzymes, the catalytic activity of rye flour is a lot higher than wheat flour6. Rye flour has large amounts of non-starch carbohydrates called pentosans (arabinoxylan and arabinogalactan), a large polymer of pentoses, mainly xylose, but also can include arabinose molecules located on cell wall, playing an important role in the viscous prop- erties of the rye flour mass that lead to the production of a characteristic bread7. Classically, the pentosan/starch ratio is taken in account as an important factor in the quality of the rye products8, but more recently, new evidence indicates that the baking properties of rye flour not only depend on total arabinoxylan content but also on arabinoxylan class present in the flour. Water-extractable arabinoxylan, the major viscos- ity factor in rye grain, had an overall positive effect, whereas water-unextractable arabinoxylan was deleterious to the baking quality of rye flour.

Numerous studies have shown that certain whole grain cereal foods rich in DF, e.g. barley kernel products, have the potential to improve important measures of cardiometabolic risk and beneficially affect appetite regulatory hormones in a semi-acute perspective, i.e. 10.5–16 h after consumption, compared to white wheat flour based bread (WWB)9. Additionally, rye-based products have previously shown potential

to improve acute postprandial appetite ratings (wholegrain flour- and bran based)10,11 and sustained appetite ratings after a 3 weeks intervention with rye porridge12 in healthy subjects. In addition, acute postprandial studies in healthy subjects have shown that rye products appear to lower the glucose and insulin response13,14 and decrease voluntary food intake at a subsequent meal, i.e. 4.5 h after the rye-based breakfast when compared to WWB14.

This scientific evidence supports rye bread and stevia are healthy and functional products with greater nutritional value for consumers, since rye flour is rich in vitamins and has beneficial health properties, being this food an essential product to contribute to the health of those who consume this kind of products15.. In this regard, the aim of this research is to design a functional bread with good organoleptic features maintaining an excellent nutritional profile with an affordable price by the reduction (or replacement) of the wheat flour6 and adding stevia as sweetener.

Methodology

Table 1 shows formulations of rye bread made after rye flour and stevia, according different studies about elaboration of bread from rye flour and stevia with better physicochemical and sensory characteristics16-21.

Table 1. Formulations for preparing bread made after rye flour and stevia with a 1Kg base

TTO	Ingredients (1000g)		
	Rye flour (g)	Wheat flour (g)	Stevia (g)
1	500	500	0.8
2	600	400	
3	800	200	
Control	0	1,000	

The physicochemical and microbiological analyses were based on the NTC 1363 for bread. The determination of moisture or volatile substances at 105°C was based on the loss of weight suffered by food when heated at 105°C. This value besides having water also included volatile substances found in the food (test done by duplicate)22. For determination of proteins, the analysis was carried out using Kjeldahl method that evaluates the content of total nitrogen in the sample, af- ter being digested with sulphuric acid in the presence of a mercury or selenium catalyst.

For fat determination, due to the insolubility of fatty substances in water and its immiscibility with it, the fat extraction from the raw materials was conducted disregarding water. The fat was extracted based on its miscibility in organic solvents, which in turn, are insoluble in water and immiscible with it. The removal of a sample previously dehydrated in an oven, is done using Soxhlet equipment with n-Hexane; later, the dissolvent is eliminated and the dry extract, which represents the lipids in the sample, was determined gravimetrically23. Fiber was determined using Soxhlet method. This method en tables to determine the fiber content in the sample, after being digested with solutions of sulfuric acid and sodium hydroxide, and burning the residue. The weight difference after the burning process indicates the amount of the fiber present.

Microbiological analysis was using total count of aerobic mesophyll, the inoculation was carried out using Agar SPC (Standard Plate Count Agar, Oxoid)24, from the dilutions previously described, and then were incubated at 35±2°C for 24- 48 hours. For the total count of total coliforms, inoculation was carried out using Violet Red Bile Agar (VRBA)25 from the dilutions previously described, and were then incubated at 35±2°C for 24-48 hours. Escherichia coli count, the inocultion was carried out using EMB agar (lactose agar, eosin- methylene blue agar, Oxoid) (ABRV) from the dilutions previously described, and were then incubated at 44°C for 24-48 hours. For counting molds and yeasts, 0.1 ml of the corresponding dilution was inoculated on the surface of a PDA Agar plate (Agar potato dextrose, Oxoid)26, then it was set aside to absorb and incubate at 25°C for 3 to 5 days.

For sensory analysis, the sensory acceptability of the final product was evaluated using a 6-point hedonic scale, 1 (I don't like it at all) and 6 (I love it) for five sensory characteristics. Additionally, two open questions were added to evaluate the personal appreciation of the respondents. The results were statistically treated through normal variance analysis (ANOVA) and post-hoc minimum significant difference test was used to discern the results of the ANOVA test. The previous analyses were carried out using the SPSS statistical software package version 19.0.

Results and discussion

Design of bread´s formulation

50% wheat flour and 50% of rye flour were used for the for- mula, the solid raw materials were mixed, and then the liquid materials were added, the kneading process was done for 10 minutes. In the following phase the bread was weighted and shaped, then it was set aside for 30 minutes and baked at 220°C for 30 minutes, the cooling time took 30 minutes, and finally the product was presented for applying the corresponding surveys. This formula was the one selected since the remaining formulations (60/40% and 80/20%), were rejected in the sensory process since rye flour has a compound called red cyclonic, which gives the bitter and pungent taste to the flour27. In the baking, the temperature used was 220°C /30 minutes similar rank was obtained6 who prepared bread with different types of flour (rye, corn, banana, wheat).

The functional ingredients used in the preparation were: oat- meal, quinoa, soy, flaxseed, etc, where butter was replaced by solid vegetable oil and sugar by stevia, same ingredients as oats, stevia and vegetable oils were used in the production of functional bakery products to contribute to the health care28. Similar studies carried out evaluated the substitution of wheat flour by rye, rice or quinoa for elaborating bread, adding salt to the dough, which generates health problems and changes in the final organoleptic perception of the product29.

Bread is obtained by many different procedures. The process applied particularly depends on several factors, including tradition, quality, cost and type of available energy, the type of desired bread and the time that must elapse between the cooking and its consumption30,31. During kneading, there is an increase in the volume which is produced in the first place by the contact with oxygen and by the incorporation of yeast, producing a small fermentation, since the metabolism of sugars free of flour stars. An increase of the temperature of the dough is also observed during the kneading (from 24°C to 28°C), as a result of the friction between the particles of the flour and the friction with the blades and the walls of the mixer, favoring the moistening processes, since the surface exposed to the aqueous phase is constantly renewed. Due to the action of the kneader the particles decrease their size and hydrate, specially their components: starch and proteins, increasing at the same time the resistance to the extension until reaching a maximum that corresponds to the time of the dough development8 The effects of the heat are observed through physical and chemical changes in a baking dough during cooking, including the increase in the volume of the alveoli, saturation and coagulation of proteins, gelatinization of the starch, formation of the cortex, browning reactions, water evaporation and development of porous structure32,33.

Heat application transforms a mature dough, hard to digest and with short duration to an attractive product light and porous texture, digestible, with durability and optimal conditions for its consumption33, 34. When introducing the dough to the oven, an increase in the volume is produced (leap of oven); during this time, the dough should have a viscosity and enough extensibility to reach the final swelling of the starch, which increases the strength of the dough structure. At the same time, gas is generated inside the dough, which expands and part of this gas is held to produce the solidification of an elastic film around 8 each bubble of gas. The whole of this development determines the final volume of the loaf of bread33,35. During the initial cooking phase, the outer layers of the dough increase their temperature to a level close to 60°C to 70°C. The surface reaches 100°C within the 3 first minutes. On the contrary, the heat transfer of the center of the crumb is relatively slow; this is due to the rapid warming of the dough surface to 100°C, generating a gradient of temperatures between the surface of the dough and the center of the crumb from 60 to 70°C. Then, the heat transfer occurs from the out- side to the inside, the moisture free of dough evaporates in the so-called "evaporation zone" as soon as the dough layers reach 100°C or more.

The gelatinization corresponds to the swelling capacity (in- crease of volume) that starch granules have in the presence of water and by effect of the temperature increment33,36. The temperature inside the dough increases from 30°C to 70°C, causing changes in the temperature range with the time. The increase in the temperature from 30°C to 40°C accelerates the swelling of the starch, the enzymatic activity and the growth of yeasts. All of the fermentable sugars continue being fermented by the action of the yeast enzymes, until reaching a temperature close to 60°C. The starch begins to gelatinize at 55°C and continues until 65°C, during this period an intense activity of the enzymes α and β amylases is produced, generating new fermentable sugars. The range of temperature for the coagulation of the protein is from 50 to 70°C, depending on the molecular weight. When 50ºC to 60ºC is reached in the center of the dough, all CO_2 generated contributes to the expansion of the dough. The thickness of the crust on the surface increases at approximately 60°C, losing its elasticity and observing the first signs of browning33,35.

During the final phase, approximately at minute 14, the temperature chosen of the oven (from 200° to 220°C) keeps constant. The interior of the dough is transformed into a structure of baked crumbs from the outside layers to the center. When the temperature of the crust reaches 170°C to 200°C the crust acquires the typical brown coloration due to browning reactions, and the aromatic substances of bread form, mainly produced by the Maillard reaction (between the reducing sugar and the amine group, which distinguishes from the caramelization where only sugars participate) in the region of the cortex. In the final phase of the cooking process the oven temperature remains constant, forming the crust and producing the desired color intensity of the crust33,35.

Physicochemical analysis

The following results were obtained in the physicochemical analysis, fiber 35.8, moisture 35.65 and 12.2 of protein, ac- cording to NTC 1363 norm this product is classified as soft bread with fiber.

Table 2. Physicochemical parameters of the rye bread and stevia (50/50%) Parameter NTC 1363 (Soft bread- Fiber) Sample of rye bread and stevia (50/50%) Min Max Moisture (% m/m) 20 40 35,65 Ashes (%) - - 2,24 Fat (g/100 g flour) 6 18 7,30 Raw fiber (%) - - 35,80 Protein (%) 9 - 12,20

Source: Quality laboratory, Universidad de Pamplona

As observed in the table, the results of the moisture, ashes, fat, raw fiber and protein of the bread with rye and stevia at (50/50%) meets the NTC 1363 standard for soft breads in Colombia. In the elaboration of bread with amaranth, the results of fiber and protein were relatively low in fiber, 11% and high in protein, 18% compared with the rye bread and stevia37, which obtained more fiber than protein percentage, because the amaranth is an herb with many nutritional benefits and is used as food for astronauts.

Microbiological analysis

As shown in Table 3 the microbiological analysis of rye bread and stevia showed that a loaf of bread is suitable for the human consumption.

Table 3. Microbiological analysis of rye bread and stevia Sample Fecal coliforms Fecal coliforms Molds and yeast Rye bread (50%) <3NMP-100mL <3NMP-100mL 65UFC/g NTC 1363 <3NMP-100mL <3NMP-100mL 1*10 2 UFC/g

Source: Quality laboratory, Universidad de Pamplo

Escherichia coli (E. coli) is a bacterium found in the digestive system of animals and humans, although these are usually harmless, some E. coli are pathogenic and can contaminate food, water and the environment38.

Likewise, the safety indicators must have association back- ground with the pathogen whose presence must indicate and be present every time it appears. The most commonly used indicators are the coliforms, usually represented by four genres of the Enterobacteriaceae family: Enterobacteriaceae: Citrobacter, Enterobacter, Escherichia and Klebsiella39. It is a group of facultative, aerobic and anaerobic, gram-negative bacteria that do not form spores, and ferment the lactose at 37°C in 48 hours, with β-galactosidase enzyme, negative oxidase and short bacilli cellular shape40. These are widely distributed in the nature, and can be found in water, soil and plants, and are part of the intestinal flora of humans and animals41,42. The fecal coliform bacteria related to the intestinal flora have the particularity of being thermotolerant, these can multiply at 44°C, can ferment the lactose, distinguishing them from the rest called total coliforms43.

Molds and yeasts are indicators of cleaning and disinfection, and potential microorganisms of food contamination. Fungi and yeasts are widely distributed in the environment; these can be found as normal flora of a food, or as contaminants in bad sanitized equipment. Certain species of fungi and yeasts are useful in the preparation of some foods, but may also cause the decomposition of other foods.

Due to its slow growth and low competitiveness, fungi and yeasts occur in foods where bacterial growth is less favorable. These conditions may be due to: low levels of pH, low moisture, high content of salt or carbohydrates, low storing temperature, and the presence of antibiotics or food exposure to radiation. Therefore, it can be a potential problem in fermented dairy foods, fruits, fruit beverages, spices, oil seeds, grains, cereals and derivatives and food with intermediate moisture such as jams, small boxes, and spices25.

Sensory analysis

In the sensory analysis of rye bread and stevia, people who were surveyed stated to like the taste, texture and presentation of the product.

Concluding remarks

Bread is a staple food part of the traditional diet in Europe, Middle East, India and America, which is prepared by baking a dough mainly made with cereal flours and sometimes with yeast (fermented bread) or without yeast (unleavened bread)6. In this regards, pastries and bakery products is the fastest growing category in food industry, accounting for al- most 80% of absolute volume growth over 2008-2013. Product innovation and increased penetration in fast growing regions such as Asia Pacific and Latin America are the main drivers of this increase. However, it is important to note that bread volumes consumption is declining in mature markets in the western hemisphere. To boost sales, manufacturers are looking into merging taste and health through adding fiber and vegetable or a mix of protein and fiber or more recently fat/sugar replacers with functional compounds like betaglucans, pentoxans, ω-3 polyunsaturated fatty acids, steviosides and rebaudiosides, polyphenols.

Rye bread with stevia is a product developed according to the demands of the NTC 1363 norms, complying with the physicochemical and microbiological parameters required by the existing legal regulations, where the results classified the product as a soft bread with fiber, without the presence of microorganism or bacteria that could harm the consumer; additionally, the sensory analysis showed that the product was accepted by the people surveyed, where the most standing features were the presentation, texture and taste of the bread.

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