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A CROSS-COUNTRY CONSUMERS' STUDY ON THE ACCEPTANCE OF GRISSINI ENRICHED WITH WHEY

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Abstract

The study aimed to valorize the whey resulting from dairy technology by its incorporation in grissini food products. It aimed to evaluate the consumers' acceptability of the new product and the market potential through a cross-country trial and survey conducted in parallel in Romania and Italy. Whey ultrafiltration concentrate used in the grissini recipe had high protein, fat, and sugar content. Grissini obtained by replacing water with whey were characterized by a higher protein content, total phenolic content, and antioxidant activity than the control sample made with water. Acceptance of the new product obtained was assessed with 100 panelists in Italy and 102 in Romania, respectively. Respondents answered a questionnaire and tested the grissinis. When it came to the acceptability of the samples made with whey, in both countries, the consumers showed a higher appreciation than for the control sample. A similar percentage of respondents (50% in Italy and 48% in Romania) were willing to pay 1.5 euro (8 lei)/100 g product. In general, participants in the study are keen to pay for innovative products at the same price as for conventional products.

***Key words**: grissini, protein content, total phenolic content, antioxidant activity, consumer acceptability.*

INTRODUCTION

In recent years, the consumers' demand for functional food products and the awareness of natural bioresources depletion has led to the necessity to come up with new products, nutritionally enriched. Among the high quantity of by-products resulting from the industry, there are resources that are edible and have the potential to be reintroduced into the food system.

In the dairy industry, whey, the liquid remaining after the cheese making and casein manufacture, is produced in high amounts annually, but around 50% of the whey globally obtained is not reintroduced in the food chains (Lavelli and Beccalli, 2022). This by-product from the dairy industry is an important source of bioactive compounds (Herrera-Ponce et al., 2022), being a rich source of nutritional, health, and functional compounds (Lavelli and Beccalli, 2022).

Whey contains 55% of milk's nutrients and is obtained by skimming and precipitation of proteins (casein), it is also known as the serum phase of milk. It comprises a water-soluble fraction that includes lactose, soluble minerals and whey proteins (Chandrapala et al., 2016; Guimarães et al., 2010). Whey, although it still has many nutritive compounds, is disposed of as wastewater and raises serious environmental problems (Zhao et al., 2023).

About 50% whey is treated for further processing into valuable products, and the rest is only disposed of as waste (Baldasso et al., 2011; Zandona et al., 2021). Estimates indicate that global whey production is around 200

million tons per year with a continuing upward trend as reported by Ostertag et al. (2023).
Whey is an important source of proteins and it can undergo the processes of microfiltration, ultrafiltration, and reverse osmosis with the purpose of obtaining a whey protein concentrate (Bacenetti et al., 2017). As a consequence of the fact that bakery products have a low content of proteins, studies were conducted in order to incorporate whey obtained in different forms in cereal-based products such as bread (Guiné et al., 2018; Tsanasidou et al., 2021; Ferreyra et al., 2021), grissini (Suhodol et al., 2022), biscuits (Ahmed et al., 2019), pasta (Boudalia et al., 2020). Additionally, by supplementing cereal-based products with whey, nutritionally enhanced products are obtained, which present improved technological, chemical, and sensory characteristics.
Grissini are dry oven-baked cereal-based products which have a long shelf-life due to the low water content, its packaging being important in order to prevent water absorption (Hui, 2007). Grissini are made usually with the addition of fat which can improve the palatability and sensory properties.
The objective of this study was to reuse a by-product from the cheese technology by incorporation in grissini samples and to obtain information regarding the acceptance and willingness to pay for these samples in selected countries, namely, Italy and Romania.

MATERIALS AND METHODS

Raw materials

To prepare grissini, the ingredients used were: whole-wheat flour (moisture: 14%, protein content: 13.3%, fats: 2.3%, carbohydrates: 56.2%, dietary fibers: 13.8%, from Velpitar 7 Spice SA Romania), fresh compressed yeast, salt, sesame and flax seeds, olive oil (from the market), and water/liquid whey concentrate (pH 4.7). Two grissini products were manufactured: control-using water as ingredient and sample-using whey obtained by concentration through ultrafiltration by ENEA, Italy (composition: moisture 89.60 ± 0.06%, protein: 5.50 ± 0.05%, lipid: 0.21 ± 0.01%, ash: 0.29 ± 0.08%, salt: 0.2%, sugar, lactose: 3.97 ± 0.09%, acidity: 53.50 ± 0.71%, pH value 4.17 ± 0.01).

Manufacturing recipe of grissini samples

Grissinis with whey were prepared in the pilot plant experiments of IBA Bucharest, Romania. Initially, fresh compressed yeast (50 g) and salt (100 g) were dissolved in water (1.8 L) for the control or in whey (1.7 L) for the sample and added to the solid mixture of ingredients (whole-wheat flour: 3 kg, sesame seeds: 208 g, flax seeds: 358 g, and olive oil: 350 ml). Thus, all ingredients were mixed slowly for 3min at a lower speed in the mixer (Diosna Original, Dierks & Söhne, Maschinenfabrik, Osnabrück, Germany), followed by a faster step for 5 min (step II) for dough development. Then, the dough was divided into pieces which were shaped automatically in the form of grissinis using grissini-forming equipment. The shaped grissini were placed on baking pans for proofing: at 36°C, 80% humidity, for 40 min in the M.C.E Meccanica fermenter, model DAT TECH 1, Buttapietra, Italy. Then, the products were baked at 240°C for 30 min in the Mondial Forni oven. After baking, the grissinis were allowed to cool for 1-2 hours before performing the analysis. For consumer tests in Italy, the grissini were shipped to Italy.

Grissini proximate composition

The physico-chemical composition of the samples was investigated using the following methods: (1) moisture content by the drying method (AOAC Method 925.23, AOAC 935.36, AOAC 926.12 and AOAC 927.05); (2) protein content by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 991.20 and AOAC 950.36); (3) fat content by extraction with petroleum ether under reflux conditions in a Soxhlet (AOAC Method 922.06 and AOAC 963.15); (4) ash content by gravimetric method by burning at 550°C in a furnace (AOAC Method 923.03 and AOAC 945.46); (5) acidity by titrimetric method (AOAC Method 947.05); (6) the total carbohydrate contents were assessed by subtracting the values of the moisture, protein, fat, and ash content from 100; (7) calorie contents were calculated using the following conversion factors: 9 for fat, 4 for carbohydrates, 4 for protein and 2 for fiber following the Commission Regulation no. 1169/2011. All analyses were performed in duplicate and results are expressed as mean ± standard deviation (SD).

Phenolic compounds and antioxidant activity determination

The extraction procedure for total phenolic content and antioxidant activity determination is described below.

To assess polyphenolic content and antioxidant activity, 2 g of the sample were weighed and mixed with 80% methanol. The extracts were obtained through an extraction process employing vortex equipment (Vortex Heidolph Instruments Multi Reax) for 4 h at room temperature. Following this, the resulting extracts were centrifuged for 30 min at 4°C and 10,000 rpm to remove any secondary materials (Multescu et al., 2022).

Determination of total phenolic content (TPC)

The determination of TPC was conducted using the Folin-Ciocalteu method with minor adjustments (Wojdyło et al., 2007). Briefly, a volume of 500 µl of the extract was mixed with 5 ml Folin-Ciocalteu reagent 1: 15, and 500 µl of 20% sodium carbonate. Following a 20 min incubation at room temperature, the absorbance was measured at 752 nm using a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). A standard curve was prepared by using various concentrations (0.005- 0.175 mg/ml) of gallic acid under the same condition with samples (R^2 = 0.9999). Total phenolic content was expressed as mg gallic acid equivalent/100g of sample (mg GAE/100 g).

Determination of antioxidant activity through the DPPH method

DPPH radical scavenging activity was assessed based on the reduction in DPPH radical, following the method described by Culețu et al. (2016). The reaction mixture consisted of 400 µl of sample and 6ml of DPPH radical solution, which was incubated for 20 min in the absence of light. Subsequently, the absorbance was recorded at 517 nm using a Specord 210 UV-VIS spectrophotometer (Analytic Jena, Germany). Antioxidant activity was calculated using a calibration curve (0.05-0.60 mmol/l) obtained with Trolox (R^2 = 0.9995). The results were expressed in mg Trolox/100 g of sample.

Determination of colour

The color of the grissini (ground samples) was measured with Konica Minolta Colorimeter (Spectrophotometer CM-5, Osaka, Japan). Three parameters were determined: parameter L*-the brightness of the sample on a scale from 0 to 100 (0 is black and 100 is white); parameter a*- the color of the sample on the scale from pure green to pure red (−a = green and +a = red), and parameter b*- the color of the sample on a scale from pure blue to pure yellow (−b = blue and +b = yellow). Before starting the analysis, the equipment was calibrated. The preparation of the samples consisted of filling the special glass cylinder of the device (diameter of 45 mm and height of 17 mm) with a sample without leaving air gaps. The sample thus prepared was placed on the measuring area and the glass was rotated in different positions to obtain 10 values for each color parameter. Means and standard deviation were calculated for each color parameter.

Consumers study

202 participants from two countries, namely Italy (100 consumers) and Romania (102 consumers), were recruited based on some screening criteria: to be part of the general population; the number of males and females to be balanced; to ensure a good spread of age, higher than 18 and younger than 60-year-old. Each participant completed a written consent form where they mentioned that they were available for participation, that they have no medication, have no food allergies or food intolerances.

Acceptance of the new product was measured based on product trials with tasting and on a survey. Both the trial and the survey were conducted in Italy and Romania.

The control sample and the grissini with whey were presented to the panelists at room temperature, in plastic plates, coded with three distinct digits. For testing, 20 g (2 grissini/person) of the control sample (grissini with water) and sample grissini with whey were provided to panelists. The samples were analyzed individually, randomly, and water was used as a palate cleanser.

Participants of the trial were informed regarding the composition of the products. In order to evaluate the acceptability of the product, the hedonic test was used, using a scale from 1 to 9, where 1 means "I dislike it extremely", and 9 "I like it extremely".

Survey questions

A questionnaire was designed for this study to evaluate the consumers' acceptance of the new product compared to the control sample and had 10 questions with open or closed answers. In both countries, the study was run through face-to-face interviews with easily understandable questions. All the consumers received detailed information about the study.

The respondents had to answer questions regarding their gender, age, allergies, knowledge about the benefits of whey, the overall acceptability of the two samples, the improvement that they consider should be made to the whey grissini, and the advantages or drawbacks of this kind of product. Additionally, they had to rate the samples after information regarding the nutritional composition and benefits of whey were provided. After rating the products based on the aspect and the data presented, grissini were tasted by panelists in a randomized order. Aspects related to willingness to pay for a new product obtained with whey and the price that they will pay for it were also measured.

Statistical analysis

All physical-chemical analyses were performed in duplicate and results were expressed as mean ± sd. For the statistical analysis Minitab statistical software version 20 was used and one-way analysis of variance (ANOVA) was performed to study the differences among the samples, followed by Tukey's test, at a confidence level of $p < 0.05$.

RESULTS AND DISCUSSIONS

Proximate composition, hardness and color parameters were determined for grissini samples. Moreover, we determined the total phenolic content and antioxidant activity using the DPPH method.

Proximate composition and nutritional properties of whey and grissini samples

Whey ultrafiltration concentrate was physicochemical and nutritional characterized and then was incorporated into grissini samples. The comparison between grissini made with water and grissini enriched with whey was investigated.

Images with grissini samples are presented in Figure 1.

Figure 1. Grissini samples: a - control (grissini with water) and b - grissini with whey

The proximate composition of the whey and grissini samples are presented in Table 1.

Table 1. Proximate composition of the whey and grissini samples

Parameters	Grissini – control	Grissini with whey
Acidity, °	4.5 ± 0.14b	5.9 ± 0.14a
Sugar, saccharose %	2.22 ± 0.08b	3.15 ± 0.07a
Salt, %	3.11 ± 0.01a	2.90 ± 0.01b
Lipid, %	15.43 ± 0.18a	15.35 ± 0.08a
Moisture content, %	4.40 ± 0.08b	5.82 ± 0.13a
Protein, %	16.07 ± 0.00b	17.22 ± 0.04a
Ash, %	3.58 ± 0.09a	3.37 ± 0.14a
Fiber, %	4.33 ± 0.08a	3.72 ± 0.08b
Starch, %	53.06 ± 0.11a	49.75 ± 0.18b
Carbohydrates	56.19	54.55
Energetical value, kcal	437	433
Energetical value, kJ	1834	1817

Values followed by different letters (a–b) for grissini samples are statistically different at $p < 0.05\%$.

Based on the acidity, whey can be classified as sweet whey (pH = 6.2-6.4) and acidic whey (pH = 4.6-5.0) (Ganju and Gogate, 2017). The whey used in our experiments was an acidic whey (pH = 4.17), which presented a moisture content of 89.60%, comparable with the one reported by Dushkova and Dinkov (2009) of 89.11% for whey ultrafiltrate.

The protein content of whey was 5.50%, higher than the one reported by Dushkova and Dinkov (2009) of 3.65%. The lipid, sugar, and ash content of whey was 0.21%, 3.97%, and 0.29%, respectively.

When compared with the control sample, grissini with whey have significantly higher values for acidity, sugar, moisture content and for protein content.

When adding whey to the grissini, the acidity increased from 4.5°T for control to 5.9°T for whey grissini. This could be the result of the natural acids present in whey. Similar results were reported by Suhodol et al. (2022) who replaced the water from grissini with whey and

obtained grissini with a higher acidity than the control sample.
The sugar content of the grissini with whey was significantly higher (3.15%) than the sugar content of the control sample (2.22%). The addition of whey improves water retention in the dough (Paul et al., 2022), and the moisture content of grissini with whey (5.82%) is significantly higher compared to the one determined for the control sample (4.40%).
By replacing water with whey in the recipe, the protein content of the grissini increased significantly.
No significant differences were noticed between the lipid and ash content of both control and grissini enriched with whey. When it comes to fiber content, the control sample had a higher fiber content (4.33%) than grissini made with whey (3.72%). Also, when it comes to salt content, the control had a higher content than grissini with whey.
The starch and carbohydrate content were higher for the control sample compared to the ones obtained for grissini with whey.
The difference in the energetic value between the two samples is negligible.

Total phenolic compounds estimation (TPC)
Table 2 presents the TPC of whey ultrafiltration concentrate, grissini control and grissini with whey. The polyphenolic content of whey ultrafiltration concentrate was 5.31 mg GAE/100 g. Grissini control and grissini with whey showed higher TPCs, recording close values, 95.42 mg GAE/100 g and 96.05 mg GAE/100 g, respectively.

Table 2. Phenolic content in analyzed grissini samples

Sample	TPC (mg GAE/100 g)
Whey ultrafiltration concentrate	5.31 ± 0.12
Grissini - control	95.42 ± 0.21a
Grissini with whey	96.05 ± 0.08a

Values followed by different letters (a–b) for grissini samples are statistically different at $p < 0.05\%$.

Antioxidant Activity of analyzed samples
After whey addition, the antioxidant activity of grissini increased significantly from 48.53 ± 0.11 mg TE/100 g for control to 63.29 ± 0.09 mg TE/100 g for grissini with whey.

Color analysis of samples
The addition of whey brings an advantage regarding the lightness of the bread crumbs samples. Both samples have positive values for the two-color parameters (a and b), the control sample has a slightly reddish coloration (Table 3) while the sample with added control whey has a slightly yellowish coloration.

Table 3. The results of the color analysis of the bread samples

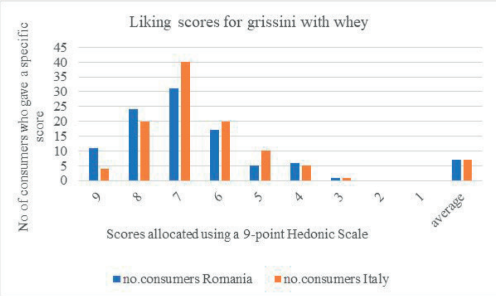
Sample	L*(D65)	a*(D65)	b*(D65)
Grissini - control	58.56 ± 0.3b	9.84 ± 0.23a	27.41 ± 0.44b
Grissini with whey	59.45 ± 0.32a	9.61 ± 0.24b	27.94 ± 0.29a

Values followed by different letters (a-b) for grissini samples are statistically different at $p < 0.05\%$.

The results are mean_standard deviation (n=10 determinations for color parameters). Values followed by different superscript letters in the same column are significantly different ($p < 0.05$).

Consumers' acceptance study
The tests of the new food product with whey were organized to evaluate the acceptance of the product prototype in two participating countries, Romania and Italy. After the consumers received detailed information about the study, the subjects were recruited from the general population based on the screening criteria mentioned before answering the questionnaire. One of these criteria was related to the allergy/intolerance to lactose (whey), sesame seeds, and gluten. In Italy, 100 consumers were recruited (56 women and 44 men, aged between 18-55+ years and overall age of 41) and completed the questionnaire. In Romania, among the 102 participants (40 men and 62 women aged between 18-55+ years and overall age of 40) to the study, 7 mentioned to have an allergy to one of the ingredients added to grissini samples or an intolerance to gluten, therefore the questionnaire ended for them without testing the grissini. 54% of Romanian participants and 62% of Italian participants declared they knew about the benefits of whey.
The overall acceptability (Figure 2) of the samples was scored using a 9-point Hedonic Scale from 9-Like Extremely to 1-Dislike Extremely.
In both countries, the whey grissini had a higher acceptability than the control.
The consumers' willingness to buy food from whey by-product was evaluated (providing a scale from: 1 euro/100 g, 1.5 euro/100 g or 2 euro/100 g). The results showed that the willingness to pay for products enriched with

by-products is a general attitude of the consumer. In Italy and Romania, a similar percentage of consumers (50% and 48%, respectively) have chosen to pay 1.5 euro/100 g product. In general, participants in the study would prefer to pay for the innovative products at the same price as for conventional products.



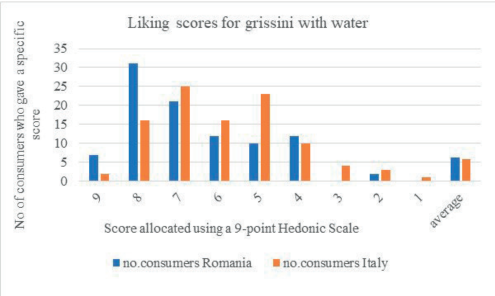


Figure 2. Overall acceptability of samples in Romania and Italy

CONCLUSIONS

The interest in reformulation of food products through incorporation of by-products started to increase. It is important to find possibilities to valorise whey in a most convenient way for reducing food waste. Incorporating whey into grissini production is a practical solution for dairy factories to valorize by-products, reduce waste, and offer healthier, sustainable food options. This approach aligns with both economic and environmental goals, contributing to a circular food system.

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