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## Development of a functional whey beverage, containing calcium, vitamin D, and prebiotic dietary fiber, and its influence on human health

### Desarrollo de una bebida funcional de suero lácteo con contenido de calcio, vitamina D y fibra dietética prebiótica y su influencia en la salud humana

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This study describes the development of a beverage made from curd whey with the addition of functional ingredients such as calcium, vitamin D3, prebiotic dietary fiber, and its influence on human health. Samples of beverages with added calcium phosphate or calcium lactate were analyzed for the selection of optimal calcium preparation. Quality attributes of beverages were evaluated in their fresh state and during storage at intervals of 15, 30, and 45 days. The beverage with calcium lactate was chosen as the one with most stable quality indices for evaluation of the effect on health status of volunteers ( $n=30$ ). Five hundred milliliters of beverage were consumed daily by the volunteers over a period of 21 days. A significant decrease in low density lipoprotein (LDL)-cholesterol ( $P<0.01$ ) and triglycerides ( $P<0.01$ ) concentrations after consumption of functional whey beverage with calcium lactate, vitamin D3, and prebiotic dietary fiber was determined.

**Keywords:** beverage; calcium; vitamin D3; dietary fiber; sensory properties; human health

Este estudio describe el desarrollo de una bebida elaborada a partir de suero de cuajada con ingredientes funcionales añadidos como el calcio, la vitamina D3, la fibra dietética prebiótica y su influencia en la salud humana. Se analizaron muestras de bebidas con fosfato de calcio añadido o lactato de calcio para la selección de la preparación óptima de calcio. Se evaluaron los atributos cualitativos de las bebidas en su estado fresco y durante el almacenamiento a intervalos de 15, 30 y 45 días. Se escogió la bebida con lactato de calcio por ser las que obtuvo los índices cualitativos más estables para la evaluación del efecto en la salud de los voluntarios ( $n = 30$ ). Los voluntarios consumieron 500 mL de la bebida durante un periodo de 21 días. Se determinó una reducción significativa de las concentraciones de colesterol LDL ( $P < 0,01$ ) y triglicéridos ( $P < 0,01$ ) después del consumo de la bebida funcional de suero lácteo con lactato de calcio, vitamina D3 y fibra dietética prebiótica.

**Palabras claves:** bebida; calcio; vitamina D3; fibra dietética; propiedades sensoriales; salud humana

### Introduction

The World Health Organization identified a deficiency of calcium to be one of the most important health problems (WHO, 2004). Calcium is essential for the structure and function of bone metabolism, for muscle functions, blood pressure, bone density, coagulation process, and releasing neurotransmitters. Some studies showed beneficial effects of dietary calcium on lipid metabolism, insulin resistance, and abdominal obesity of persons with metabolic syndrome (Jacqmain, Doucet, Despres, Bouchard, & Tremblay, 2003; Lorenzen & Astrup, 2011). The adequate intake of calcium from food products can be considered as one of the most important factors that can help senior consumers in retaining bone mass and avoiding osteoporosis. The positive effect of adequate calcium intake on different chronic diseases such as hypertension, colon cancer, breast cancer, and other disorders was also shown (Miller, Jarvis, & McBean, 2001). The sufficient absorption of calcium is also important.

The possibility of using calcium salts for fortification was analyzed for various products: apple juice (Russell et al., 2010), milk (On-Nom, Grandison, & Lewis, 2012), flat bread (Ziadeh et al., 2005), and beverages (Gonnelli et al., 2007). Different calcium salts, such as calcium carbonate, calcium citrate,

calcium sulfate (Ziadeh et al., 2005), calcium lactate, and calcium gluconate (Russell et al., 2010) were analyzed in this field. Main features of calcium preparation for usage on the industrial scale depend not only on the bioavailability of calcium in a food product (Heaney, Rafferty, Dowell, & Bierman, 2005; Perales, Barbera, Lagarda, & Farre, 2006), but also on technological factors (Schroder, Griffin, Specker, & Abrams, 2005), the cost of calcium preparation, etc. The choice of calcium salts for the fortification of food products is dependent on its compatibility with the manufacturing processes (Rafferty, Walters, & Heaney, 2007), the possible effect on the sensory attributes, and the stability characteristics of the products. Some studies have shown that calcium salts may have an unpleasant effect on the product taste (Lawless, Rapacki, Horne, & Hayes, 2003; Ziadeh et al., 2005).

Vitamin D is an essential fat-soluble vitamin needed for efficient calcium absorption. The deficiency of vitamin D has been related to hypertension, diabetes, metabolic syndrome, cancer, autoimmune, and infectious diseases (Ferguson, Laing, Marlow, & Bishop, 2015; Holick & Chen, 2008). Synthesis of vitamin D in the skin by the action of sunlight is insufficient to meet the requirements in European countries, especially, during winter months when there is little sunlight exposure. Sub-

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optimal vitamin D status has been reported in sub-groups of children and adolescents in many European countries, particularly, in winter months, indicative of inadequate vitamin D intake (Cashman, 2007; Viljakainen et al., 2006).

Prebiotics are non-digestible food ingredients that promote human health benefits, including cholesterol lowering effects, improving gastrointestinal microflora by selectively promoting the growth of probiotics, and inhibition of pathogenic microorganisms (Bielecka, Biedrzycka, & Majkowska, 2002). Prebiotics reduce blood lipids and blood pressure, increase the synthesis and absorption of nutrients and minerals, and have anticarcinogenic action (Huebner, Wehling, & Hutkins, 2007; Saier, Jr. & Mansour, 2005; Scholz-Ahrens, Ade, & Marten, 2007). Prebiotics as a functional ingredient are important for the creation of new generations of functional beverages.

Bioactive substances with health promoting features could have negative effects on the product sensory properties and acceptability as the interactions between added bioactive substances and applied technological factors or a deterioration of the used matrix components can appear during the product storage time (Cilla et al., 2011; Singh et al., 2007; Tobelmann, 2001).

Functional beverages can be produced using as a base matrix the whey from curd production. Whey contains about 50–55% total milk solids, 70% of milk sugar, 20% of milk proteins, and 70–90% of minerals and almost all water soluble vitamins, especially, vitamin B complex and vitamin C (Sinha, Radha, Prakash, & Kaul, 2007). Many easily digestible bioactive components derived from whey are under study for their ability to offer specific health benefits (Frid, Nilsson, Holst, & Bjorck, 2005; Ha & Zemel, 2003). Whey proteins as functional ingredients reflect a wide range of potential therapeutic applications. Whey proteins consist of lactoferrin, lactoperoxidase,  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, bovine serum albumin, thermostable fractions of proteose peptones, immunoglobulins, and bioactive peptides. The presence of lysine, cysteine, methionine, and cystine imparts anticarcinogenic properties to these proteins (Durham, Hourigan, Sleight, & Jhonsen, 1997; Jelacic, Bozanic, & Tratnik, 2008). Individual whey protein components and their peptide fragments stimulate the immune system, anticarcinogenic activity, and other metabolic features (Gobbetti, Stepaniak, De Angelis, Corsetti, & Di Cagno, 2002). The nutritional and functional properties of whey are of great significance for the formulation of food products (Kabašinskiėnė, Liutkevičius, Sekmokienė, Zaborskienė, & Šlapkauskaitė, 2015). Whey and whey-derived products, apart from being nutritional ingredients in various foods, can also be used as functional ingredients providing flavor, texture, color, and aeration properties in a variety of foods.

The novelty and the aim of the present study were to establish the effect of added calcium in complex with vitamin D3 and prebiotic dietary fiber on the physicochemical and sensory quality of whey-based beverages in order to select the best preparation for beverages and to evaluate the effect of developed functional beverages on human's health.

## Materials and methods

### Materials

The beverages were prepared in a curd whey–water matrix with the addition of functional ingredients such as natural prebiotic dietary fiber based on acacia gum with a soluble fiber content over 90% (Colloides Naturels International, France), vitamin D<sub>3</sub>

premix for pasteurized milk (Fortitech, Inc., Denmark), and calcium preparation. Two different calcium preparation methods were used for the fortification of the beverages: micronized tricalcium phosphate coated with soy lecithin (containing 36% of calcium) and calcium lactate (containing 14.7% of calcium) was chosen as highly bioavailable calcium preparation methods. The amount of functional ingredients added to 100 g of beverage was calculated to provide 150 mg of calcium, 0.75  $\mu$ g of vitamin D<sub>3</sub>, and 2.5 g of prebiotic dietary fiber. The curd whey was obtained from a local dairy. Sugar, pectin, flavoring compounds of tropical fruits, and carrot (Rudolf Wild GmbH & Co. KG, Germany) were also used (Table 1) in the preparation of the beverages as additional components with the aim to achieve the best nutritional and sensory properties of beverages.

### Preparation of beverages

All the components were dissolved in a mixture of curd whey and water (1:1) preheated to 40°C. The pH of the beverages was standardized (if needed) by acidifying with citric acid to pH 4.1–4.2 with the aim to enhance their stability and sensory properties during storage. Samples were pasteurized at a temperature of 80–85 °C/15–20 s, cooled down, distributed into sterile plastic bottles (500 mL) and then stored at a temperature of 4  $\pm$  2 °C until testing. The properties of the beverages were analyzed every 15 days for a 45 days period. The duration of storage (45 days) was determined in preliminary experiments (Liutkevičius, Alenčikienė, Speičienė, & Miežilienė, 2014).

Active acidity was determined using a Wissenschaftlich-Technische-Werkstätten GmbH microprocessor meter pH 538, using electrode with a Sen Tix 97 T integrated temperature sensor.

(L+) and (D-) lactic acid in the beverages were determined by a method of fermentation, where Boehringer Mannheim/R-Biopharm agent kit was used.

### Color measurements

Color parameters L\* (lightness), a\* (redness), and b\* (yellowness) as determined by CIE (Comisión Internacional de l'Eclairage) were measured using a CR-400 Chroma Meter (Konica Minolta, Osaka, Japan). A white reference plate for calibration, a D65 illuminant, a viewing angle of 0° and a measurement area of 8 mm were used. Chroma (C\*), which indicates color intensity, and hue (h\*), which signifies product color were calculated.

Table 1. The formulation of 100 g of beverages.

Tabla 1. La formulación de 100 g de las bebidas.

Ingredients	Samples		
	Control	Calcium phosphate	Calcium lactate
Whey, g	45.5	45.5	45.5
Pectin, g	0.3	0.3	0.3
Sugar, g	7.0	7.0	7.0
Flavor compound, g	1.5	1.5	1.5
Calcium phosphate, mg	0.0	416.7	0.0
Calcium lactate, mg	0.0	0.0	1020.4
Vitamin D3 premix for pasteurized milk, g	0.0	5	5
Prebiotic dietary fiber, g	0.0	2.5	2.5
Water, g	Till 100	Till 100	Till 100

### Sensory analysis

A quantitative descriptive analysis (QDA) was carried out for the assessment of the sensory properties, and sensory profile was created for each prepared beverage. A total group of seven trained assessors (female, ages 20–60 years old) having work experience of not less than 20 hours in the evaluation of various food products was used. Sensory panel orientation, training and calibration process consisted of 2 training sessions during 2 days. In the first session sensory assessors were asked to develop a list of attributes to describe the sensory attributes of the tested samples, during the second session assessors were practiced to use the scales for each selected attribute. All training and data collection sessions were held in the sensory analysis laboratory established according to International Organization for Standardization 8589 (2007) requirements. For the development of the sensory profiles, a full balanced randomized sample presentation plan with two repetitions was applied. Panel responses were collected using a computerized program (Fizz, Biosystems, France). An unstructured 150 mm line scales with 10 mm indented anchors (left – “low intensity/absent”, right – “high intensity”) were used to evaluate each sensory attribute. Scales were presented for each sample on a single screen for evaluation of the attributes of odor, texture and taste. The following attributes were evaluated: overall odor, citrus fruit odor, sour odor, non-typical odor, visual thickness, homogeneity (in the mouth), overall taste intensity, richness of taste, citrus fruit taste, acid taste, bitter taste, astringent taste, non-typical taste, and mouthcoating.

Beverages were kept in closed bottles in a refrigerator ( $4 \pm 2^\circ\text{C}$ ) until testing and were removed from refrigerator 30 min prior to a sensory evaluation which allowed the samples to equilibrate to room temperature ( $21 \pm 2^\circ\text{C}$ ). Then the samples of the beverages (approximately 20 mL) were presented to the assessors in 30 mL plastic cups, coded with three digit numbers.

Samples for the acceptability test of the beverages were kept and presented for analysis in the same way as for a descriptive analysis (see section sensory analysis). Not less than 35 consumers who usually consume such type of beverages were recruited from the local area for each testing (most of them participated in all four sessions). The consumers received a verbal instruction about the testing procedure before the test. A 15 point hedonic scale was used for evaluation of the beverages (1 – “not acceptable”, 15 – “very acceptable”).

### Study design of a biomedical test

Thirty healthy volunteers (aged between 20–24 years) were recruited for the study. The participants were asked to maintain their normal dietary and lifestyle habits throughout the study (21 day) except the 500 mL of beverage. The beverage doses (500 mL) were presented to participants in separate bottles for each day.

The participants were invited to arrive at the hospital between 7:30 a.m. and 9:00 a.m. after having fasted for 12 hours. Their blood pressure was measured twice using a manometer, pulse rate was measured once after resting supine for 5 minutes, body composition monitor was performed by “Omron BF 511”. All measurements and blood samples were taken on the first visit before the drink has been consumed and on the second visit after the last dose of beverage (on the 22nd day).

The study was approved by the Lithuanian Bioethics Committee (2012-11-29; order No. 158200-12-227-158).

### Biochemical analyses

Cholesterol and triglyceride concentrations in serum were analyzed by enzymatic colorimetric methods (Architect ci8200, Abbott, USA). A low density lipoprotein cholesterol concentration was calculated using the *Friedewald* formula. High density lipoprotein cholesterol was analyzed by the accelerator selective detergent method (Architect ci8200, Abbott, USA).

Plasma glucose concentration was analyzed by hexokinase enzymatic method (Architect ci8200, Abbott, USA). The serum insulin was measured by chemiluminescent microparticle immunoassay (Architect ci8200, Abbott, USA).

Fibrinogen concentration in blood plasma was analyzed by the Clauss coagulometric method (STA-Compact, Diagnostica Stago, France).

C-reactive protein was analyzed by latex enhanced immunoturbidimetric assay (Architect ci8200, Abbott, USA).

### Statistical methods

A sensory evaluation was performed in duplicate, instrumental measurements were performed in triplicate. The data were analyzed by analysis of variance, in cases where significant interactions were determined, multiple comparisons were made. The differences were classified by a *Duncan* multiple comparison test ( $P < 0.05$ ). SPSS (Statistical Package for Social Sciences) software, version 15.0 (Chicago, IL, USA, 2006), was used for the statistical analysis of the data.

Statistical analysis of medical data was performed using SPSS (Statistics Base 19.0). A dependent *t*-test for paired samples was carried out to examine the differences between the baseline and 21-day follow-up results. The significance level of  $P \leq 0.05$  was used for all the analyses.

## Results and discussions

### Physicochemical characteristics

Lactic acid isomers were evaluated in samples of beverages, and was detected that L(+) lactic acid, which is considered as more favorable for human health, dominates over D(-) lactic acid in all beverages (Table 2). The highest amount of L(+) lactic acid was found in the samples with calcium lactate. L(+) lactic acid is considered as more favorable for human health, (Gorbatova, 1984).

Color characteristics of beverages enriched by calcium, vitamin D3 and prebiotic dietary fiber are presented in Table 3. The color of all the beverages was conditioned by the orange color of flavor compound included in the formulations.

Table 2. Properties of beverages.

Tabla 2. Propiedades de las bebidas.

Samples of beverages	pH	Lactic acid, g/100 g	
		D-	L+
Control	$4.15 \pm 0.01^a$	$0.008 \pm 0.006^a$	$0.281 \pm 0.041^a$
Calcium phosphate	$4.12 \pm 0.02^a$	$0.005 \pm 0.005^a$	$0.249 \pm 0.090^a$
Calcium lactate	$4.13 \pm 0.02^a$	$0.012 \pm 0.006^a$	$0.593 \pm 0.590^b$

Note: <sup>a, b</sup> – mean values within each column with different superscripts are different at  $P < 0.05$

Nota: <sup>a, b</sup> – los valores promedio en cada columna con diferentes superíndices son distintos a  $P < 0,05$

Table 3. Effect of different Ca preparation added to beverages together with vitamin D3 and dietary fiber on color characteristics of samples during storage.

Tabla 3. Efecto de las diferentes preparaciones de Ca añadidas a las bebidas juntamente con vitamina D3 y fibra dietética en las características del color de las muestras durante el almacenamiento.

Characteristic	Storage	Sample		
	Duration, days	Control	Calcium phosphate	Calcium lactate
L*	0	32.67 ± 0.09 <sup>b</sup>	35.67 ± 0.01 <sup>c</sup>	31.90 ± 0.04 <sup>a</sup>
	15	33.38 ± 0.10 <sup>b</sup>	37.50 ± 0.09 <sup>c</sup>	32.50 ± 0.04 <sup>a</sup>
	30	33.33 ± 0.04 <sup>b</sup>	36.70 ± 0.06 <sup>c</sup>	32.77 ± 0.00 <sup>a</sup>
	45	33.21 ± 0.04 <sup>b</sup>	36.09 ± 0.01 <sup>c</sup>	32.19 ± 0.03 <sup>a</sup>
a*	0	0.63 ± 0.03 <sup>a</sup>	1.51 ± 0.04 <sup>b</sup>	0.64 ± 0.04 <sup>a</sup>
	15	0.80 ± 0.08 <sup>a</sup>	2.19 ± 0.07 <sup>b</sup>	0.78 ± 0.04 <sup>a</sup>
	30	0.76 ± 0.07 <sup>a</sup>	2.13 ± 0.11 <sup>b</sup>	0.93 ± 0.07 <sup>a</sup>
	45	1.51 ± 0.05 <sup>b</sup>	2.06 ± 0.05 <sup>c</sup>	1.27 ± 0.08 <sup>a</sup>
b*	0	23.73 ± 0.17 <sup>a</sup>	25.24 ± 0.12 <sup>b</sup>	23.39 ± 0.02 <sup>a</sup>
	15	24.42 ± 0.30 <sup>b</sup>	26.40 ± 0.04 <sup>c</sup>	23.46 ± 0.09 <sup>a</sup>
	30	24.58 ± 0.25 <sup>b</sup>	25.26 ± 0.12 <sup>c</sup>	23.04 ± 0.06 <sup>a</sup>
	45	24.57 ± 0.21 <sup>b</sup>	25.51 ± 0.02 <sup>c</sup>	23.23 ± 0.08 <sup>a</sup>
h*	0	1.54 ± 0.00 <sup>b</sup>	1.51 ± 0.00 <sup>a</sup>	1.54 ± 0.00 <sup>b</sup>
	15	1.54 ± 0.00 <sup>b</sup>	1.49 ± 0.01 <sup>a</sup>	1.54 ± 0.00 <sup>b</sup>
	30	1.54 ± 0.00 <sup>b</sup>	1.50 ± 0.00 <sup>a</sup>	1.53 ± 0.00 <sup>b</sup>
	45	1.51 ± 0.00 <sup>b</sup>	1.49 ± 0.01 <sup>a</sup>	1.52 ± 0.00 <sup>b</sup>
C*	0	23.74 ± 0.17 <sup>a</sup>	25.29 ± 0.12 <sup>b</sup>	23.40 ± 0.02 <sup>a</sup>
	15	24.43 ± 0.30 <sup>b</sup>	25.49 ± 0.04 <sup>c</sup>	23.47 ± 0.09 <sup>a</sup>
	30	24.59 ± 0.25 <sup>b</sup>	25.33 ± 0.13 <sup>c</sup>	23.26 ± 0.06 <sup>a</sup>
	45	24.61 ± 0.21 <sup>b</sup>	25.60 ± 0.02 <sup>c</sup>	23.26 ± 0.08 <sup>a</sup>

Note: a, b, c – mean values within each row with different superscripts are different at  $P < 0.05$ Nota: a, b, c – los valores promedio en cada fila con diferentes superíndices son distintos a  $P < 0,05$ 

The lightness (L\*) of the beverage samples with calcium lactate decreased ( $P < 0.001$ ) in comparison with the control beverage, and the same tendency was noticed for their yellowness (b\*) ( $P < 0.001$ ), and redness (a\*) ( $P < 0.001$ ). This explains why the chroma (C\*) value was lower than that of the control sample. This tendency was recorded during the entire storage period.

The addition of calcium phosphate together with two other functional ingredients increased the values of lightness ( $P < 0.001$ ), redness ( $P < 0.001$ ), and yellowness ( $P < 0.001$ ) in comparison with the control sample. The effect of functional ingredients on chroma ( $P < 0.001$ ) and hue ( $P < 0.05$ ) values was also significant. During storage, some variation in color characteristics values was determined, but a tendency of increased lightness, redness and decreased hue values in comparison with fresh samples was noticed for all samples of the beverages.

Changes in color characteristics influenced by the addition of functional ingredients were statistically significant ( $P < 0.05$ ), but not significant enough to have a negative effect on the product color changes as C\* value varied at an interval from 23 to 25 and hue value varied at an interval from 1.49 to 1.53, which is quite similar for the human eye.

### Sensory properties

Formulation of the beverage (i.e. calcium preparation), showed a significant effect on various sensory properties of the beverages, such as overall odor, odor of citrus fruit and freshness of odor, homogeneity, intensity of overall taste, taste of citrus fruits, sourness, bitterness, astringency, and mouthcoating (Table 4). In general, the freshness of odor of the beverages with calcium preparation showed a tendency to be lower than in a control

sample. A visual evaluation of the samples by the panel showed that the effect of different composition on the samples color characteristics was not significant (data not presented, as no significant changes were determined depending from composition or storage time).

The addition of calcium phosphate decreased the intensity of overall odor ( $P < 0.05$ ) and the freshness of odor became less expressed ( $P < 0.05$ ) in beverage during all tested period (45 days). Visual homogeneity of fresh samples (0 days) was not affected by calcium phosphate and other two functional ingredients. The negative effect of added ingredients on homogeneity of beverage was noticed after 15 days, as the beverage was evaluated as less homogeneous than the control sample. The overall taste of the beverage became less intensive after functional ingredients were added, what was affected by less expressed sourness and taste of citrus fruit of the beverage. Some weak note of astringency was found in the beverages when functional ingredients with calcium phosphate were added. A low level of increased bitterness was detected in the sample with calcium phosphate compared with the control sample during all tested period. The presence of the weak non-typical taste was noticed when compared with the control sample only at the end of beverage storage (45 days).

Significant changes in taste and odor attributes of the beverages with calcium phosphate had no significant effect on the odor acceptability, but it decreased acceptability of taste, particularly, at the end of storage. The overall acceptability and acceptability of the texture of beverage with calcium lactate was lower than that of the control sample at the end of the storage (Table 4).

The addition of calcium lactate increased astringency ( $P < 0.001$ ) and decreased freshness of odor of the fresh



Table 4. Effect of different Ca preparation added to beverages together with vitamin D3 and dietary fiber on sensory properties and acceptability of samples during storage.

Tabla 4. Efecto de las diferentes preparaciones de Ca añadidas a las bebidas juntamente con vitamina D3 y fibra dietética en las propiedades sensoriales y aceptabilidad de las muestras durante el almacenamiento.

Attribute	Control				Calcium phosphate				Calcium lactate			
	Age of sample, days											
	0	15	30	45	0	15	30	45	0	15	30	45
Overall odor	112.0 <sup>b</sup>	117.1 <sup>b</sup>	116.2 <sup>b</sup>	115.1 <sup>b</sup>	110.8 <sup>a</sup>	111.4 <sup>a</sup>	107.4 <sup>a</sup>	105.5 <sup>a</sup>	111.6 <sup>ab</sup>	110.7 <sup>a</sup>	106.8 <sup>a</sup>	110.8 <sup>a</sup>
Citrus fruits odor	99.9 <sup>a</sup>	102.0 <sup>b</sup>	100.6 <sup>b</sup>	105.0 <sup>b</sup>	96.1 <sup>a</sup>	94.9 <sup>a</sup>	88.2 <sup>a</sup>	94.6 <sup>a</sup>	99.4 <sup>a</sup>	98.5 <sup>ab</sup>	89.8 <sup>a</sup>	99.3 <sup>a</sup>
Freshness of odor	101.1 <sup>b</sup>	87.6 <sup>b</sup>	93.1 <sup>b</sup>	82.5 <sup>b</sup>	93.3 <sup>a</sup>	78.4 <sup>a</sup>	80.1 <sup>a</sup>	67.6 <sup>a</sup>	95.1 <sup>a</sup>	80.4 <sup>a</sup>	80.5 <sup>a</sup>	75.3 <sup>a</sup>
Sour odor	87.6 <sup>a</sup>	77.7 <sup>a</sup>	71.2 <sup>a</sup>	64.5 <sup>b</sup>	85.0 <sup>a</sup>	63.8 <sup>a</sup>	60.1 <sup>a</sup>	57.7 <sup>ab</sup>	80.7 <sup>a</sup>	76.4 <sup>a</sup>	69.6 <sup>a</sup>	54.7 <sup>a</sup>
Non-typical odor	18.0 <sup>a</sup>	26.4 <sup>a</sup>	13.8 <sup>a</sup>	19.3 <sup>a</sup>	21.4 <sup>a</sup>	21.0 <sup>a</sup>	18.4 <sup>a</sup>	24.5 <sup>a</sup>	19.0 <sup>a</sup>	21.4 <sup>a</sup>	18.7 <sup>a</sup>	19.8 <sup>a</sup>
Thickness visual	39.7 <sup>a</sup>	29.8 <sup>a</sup>	31.9 <sup>a</sup>	25.1 <sup>a</sup>	32.7 <sup>a</sup>	37.2 <sup>a</sup>	36.0 <sup>a</sup>	25.3 <sup>a</sup>	28.8 <sup>a</sup>	28.2 <sup>a</sup>	30.9 <sup>a</sup>	23.8 <sup>a</sup>
Homogeneity	115.5 <sup>a</sup>	125.2 <sup>b</sup>	121.3 <sup>b</sup>	127.3 <sup>b</sup>	113.5 <sup>a</sup>	106.8 <sup>a</sup>	104.8 <sup>a</sup>	103.2 <sup>a</sup>	115.5 <sup>a</sup>	115.5 <sup>ab</sup>	117.3 <sup>ab</sup>	127.3 <sup>b</sup>
Overall taste	115.5 <sup>b</sup>	124.3 <sup>b</sup>	113.8 <sup>b</sup>	119.8 <sup>b</sup>	111.3 <sup>a</sup>	111.9 <sup>a</sup>	111.6 <sup>a</sup>	112.6 <sup>a</sup>	113.4 <sup>ab</sup>	122.7 <sup>ab</sup>	112.8 <sup>ab</sup>	118.3 <sup>ab</sup>
Richness of taste	94.5 <sup>a</sup>	106.0 <sup>a</sup>	104.7 <sup>a</sup>	103.5 <sup>a</sup>	92.1 <sup>a</sup>	97.9 <sup>a</sup>	94.3 <sup>a</sup>	89.8 <sup>a</sup>	93.3 <sup>a</sup>	90.0 <sup>a</sup>	92.5 <sup>a</sup>	95.0 <sup>a</sup>
Citrus fruit taste	104.1 <sup>b</sup>	108.6 <sup>b</sup>	108.8 <sup>b</sup>	106.1 <sup>b</sup>	93.4 <sup>a</sup>	99.0 <sup>a</sup>	85.0 <sup>a</sup>	89.0 <sup>a</sup>	102.6 <sup>b</sup>	106.3 <sup>b</sup>	99.2 <sup>b</sup>	99.7 <sup>b</sup>
Sourness	84.3 <sup>b</sup>	88.2 <sup>b</sup>	80.2 <sup>b</sup>	75.3 <sup>b</sup>	66.4 <sup>a</sup>	67.9 <sup>a</sup>	66.1 <sup>a</sup>	64.1 <sup>a</sup>	93.7 <sup>b</sup>	96.7 <sup>b</sup>	82.9 <sup>b</sup>	93.0 <sup>b</sup>
Sweetness	67.0 <sup>a</sup>	72.6 <sup>a</sup>	60.0 <sup>a</sup>	70.5 <sup>a</sup>	72.3 <sup>a</sup>	66.4 <sup>a</sup>	66.1 <sup>a</sup>	72.7 <sup>a</sup>	69.6 <sup>a</sup>	61.8 <sup>a</sup>	55.6 <sup>a</sup>	55.3 <sup>a</sup>
Bitterness	23.4 <sup>a</sup>	22.6 <sup>a</sup>	25.0 <sup>a</sup>	20.6 <sup>a</sup>	30.9 <sup>b</sup>	24.3 <sup>b</sup>	28.0 <sup>b</sup>	30.3 <sup>b</sup>	29.4 <sup>a</sup>	24.4 <sup>b</sup>	34.8 <sup>b</sup>	31.31 <sup>b</sup>
Non-typical taste	18.0 <sup>a</sup>	26.8 <sup>a</sup>	18.5 <sup>a</sup>	20.2 <sup>a</sup>	21.6 <sup>a</sup>	27.7 <sup>a</sup>	21.9 <sup>a</sup>	33.9 <sup>b</sup>	20.1 <sup>a</sup>	27.6 <sup>a</sup>	20.4 <sup>a</sup>	27.0 <sup>ab</sup>
Astringency	19.2 <sup>a</sup>	20.8 <sup>a</sup>	27.6 <sup>a</sup>	25.5 <sup>a</sup>	22.0 <sup>b</sup>	27.6 <sup>b</sup>	29.7 <sup>b</sup>	33.1 <sup>b</sup>	27.1 <sup>b</sup>	33.9 <sup>b</sup>	33.3 <sup>b</sup>	33.5 <sup>b</sup>
Mouthcoating	21.6 <sup>a</sup>	25.9 <sup>a</sup>	31.9 <sup>a</sup>	26.8 <sup>a</sup>	23.8 <sup>a</sup>	36.9 <sup>b</sup>	42.9 <sup>b</sup>	43.5 <sup>b</sup>	22.0 <sup>a</sup>	31.8 <sup>b</sup>	35.3 <sup>b</sup>	32.4 <sup>b</sup>
Aftertaste	87.9 <sup>a</sup>	82.6 <sup>a</sup>	82.0 <sup>a</sup>	70.8 <sup>a</sup>	84.7 <sup>a</sup>	81.7 <sup>a</sup>	84.1 <sup>a</sup>	71.0 <sup>a</sup>	83.1 <sup>a</sup>	85.6 <sup>a</sup>	83.8 <sup>a</sup>	74.2 <sup>a</sup>
Odor acceptability	115.6 <sup>aA</sup>	112.0 <sup>aA</sup>	113.1 <sup>bA</sup>	114.9 <sup>bA</sup>	114.3 <sup>aA</sup>	112.6 <sup>aA</sup>	105.7 <sup>aA</sup>	105.9 <sup>aA</sup>	116.4 <sup>aA</sup>	117.0 <sup>aA</sup>	106.8 <sup>bA</sup>	112.6 <sup>bA</sup>
Taste acceptability	112.2 <sup>aA</sup>	113.5 <sup>aA</sup>	109.3 <sup>aA</sup>	113.2 <sup>bA</sup>	104.1 <sup>ab</sup>	106.8 <sup>ab</sup>	100.0 <sup>ab</sup>	96.3 <sup>aA</sup>	109.2 <sup>aA</sup>	104.2 <sup>aA</sup>	98.5 <sup>aA</sup>	99.9 <sup>abA</sup>
Texture acceptability	118.6 <sup>aA</sup>	120.7 <sup>bA</sup>	114.3 <sup>bA</sup>	114.3 <sup>bA</sup>	115.2 <sup>ab</sup>	112.6 <sup>ab</sup>	108.9 <sup>aAB</sup>	99.4 <sup>aA</sup>	118.2 <sup>aA</sup>	117.7 <sup>abA</sup>	114.9 <sup>abA</sup>	107.4 <sup>abA</sup>
Overall acceptability	115.8 <sup>aA</sup>	106.8 <sup>aA</sup>	112.5 <sup>aA</sup>	114.5 <sup>bA</sup>	109.8 <sup>ab</sup>	109.9 <sup>ab</sup>	102.9 <sup>aAB</sup>	97.8 <sup>aA</sup>	115.4 <sup>bA</sup>	107.9 <sup>aA</sup>	103.2 <sup>aA</sup>	105.9 <sup>abA</sup>

Note: <sup>a, b</sup> – mean values within each row for same age of sample with different superscripts are different at  $P < 0.05$ <sup>A,B</sup> – mean values within each row for same sample during storage with different superscripts are different at  $P < 0.05$ Nota: <sup>a, b</sup> – los valores promedio en cada fila de las muestras con la misma edad con diferentes superíndices son distintos a  $P < 0.05$ <sup>A,B</sup> – los valores promedio en cada fila para la misma muestra durante el almacenamiento con diferente superíndice son distintos a  $P < 0.05$ 

beverage (Table 4). During tested period (0–45 days) this effect remained clearly expressed. The effect of added ingredients on the other sensory properties of the beverage was not significant for fresh samples, but after 15 days intensity of overall odor was lower and intensity of bitterness higher compared to control sample. Beverage with calcium lactate remains homogeneous all tested period.

No difference in mouthcoating was noticed for fresh samples (0 days). After 15 days of storage, more intensive mouthcoating feeling was perceived in beverages with functional ingredients compared to a control sample.

Sometimes consumers agree to compromise sensory attributes for other perceived benefits, such as nutritional value or country of origin of the products (Kähkönen & Tuorila, 1999; Menrad, 2003). However, if the sensory attributes of the product do not meet consumers' expectations, it is unlikely that such product will be used again (McIlveen & Buchanan, 2001). Nowadays, consumers can find very different functional products with the same ingredients and the same effect on health, so sensory properties of products should meet consumers' expectations in order to encourage consumers to include the product in their diet.

In this regard, the addition of calcium lactate together with vitamin D3 and dietary fiber did not show a negative effect on the sensory properties and acceptability of the beverage. Beverage with added calcium lactate was more similar to the control sample than sample with calcium phosphate during all tested period. These findings suggest calcium lactate as a more suitable calcium preparation for biomedical testing of the beverage.

### Medical testing

Results (Table 5) showed that the consumption of the beverage enriched with calcium lactate, vitamin D3 and prebiotic dietary fiber decreased the concentration of low density lipoprotein

Table 5. Serum levels of lipids, carbohydrates and other blood characteristics of the group of volunteers ( $n = 30$ ) before and after beverage supplementation.Tabla 5. Los niveles de suero de los lípidos, los carbohidratos y otras características sanguíneas del grupo de voluntarios ( $n = 30$ ) antes y después de cada suplemento de bebida.

Parameter	Before	After	<i>t</i>	<i>P</i>
Total cholesterol (mmol/L)	4.69 ± 0.91	4.48 ± 0.73	2.075*	0.049
HDL-cholesterol (mmol/L)	1.51 ± 0.36	1.53 ± 0.34	-0.736	0.469
LDL-cholesterol (mmol/L)	2.82 ± 0.74	2.53 ± 0.58	3.165**	0.004
Triglycerides (mmol/L)	0.91 ± 0.42	0.77 ± 0.31	-3.049**	0.006
Insulin (pmol/L)	57.14 ± 27.62	65.48 ± 24.84	-1.740	0.095
Fasting glucose (mmol/L)	5.00 ± 0.40	4.91 ± 0.42	1.195	0.244
Fibrinogen (g/L)	2.94 ± 0.52	2.98 ± 0.74	-0.292	0.773
CRP (mg/L)	0.54 ± 0.45	1.28 ± 2.13	-1.936	0.065

Note: HDL – high density lipoprotein; LDL – low density lipoprotein; CRP – C-reactive protein.

\*  $P \leq 0.05$ , \*\*  $P < 0.01$ 

Nota: HDL – lipoproteína de alta densidad; LDL – lipoproteína de baja densidad; CRP – proteína C reactiva.

\*  $P \leq 0.05$ , \*\*  $P < 0.01$

(LDL) cholesterol by 10.3% ( $P < 0.01$ ). A tendency ( $P = 0.049$ ) for decrease of total cholesterol concentration after consumption of the beverage was also observed (Table 5). This change in concentrations shows a beneficial effect on improving lipid profiles of the group of volunteers.

Prebiotics are effective in improving lipid profiles, including the reduction of total cholesterol, LDL-cholesterol, triglycerides and increasing of HDL-cholesterol in blood plasma (Begley, Hill, & Gahan, 2006; Ooi & Liang, 2010). Cholesterol lowering effect of prebiotics can be explained by the enzymatic deconjugation of bile acids by bile salt hydrolase of probiotics. Bile, a water-soluble end product of cholesterol in the liver, is stored and concentrated in the gallbladder, and released into the gastrointestinal tract upon ingestion of food. Deconjugated bile acids are less soluble and absorbed by the intestines, leading to their elimination in the feces. Cholesterol is used to synthesize new bile acids resulting in lowering of serum cholesterol. But still little information is available on the effective dosage of prebiotics needed to exert hypocholesterolemic effects (Lay-Gaik & Min-Tze, 2010).

Supplementary calcium that was included in the beverage composition could also have impact on blood lipids profile. A beneficial effect on the lipid profile was shown in the studies of both animals and humans (Vaskonen, 2003). It was shown that supplementary calcium has a cholesterol lowering effect, which can be explained, at least partly, by the formation of insoluble calcium fatty acids soaps and/or the binding of bile acids as an increase in fecal excretions of fat and bile acids was reported (Christensen et al., 2009; Lorenzen & Astrup, 2011).

The present study has also shown that developed beverage had significant effect on the decreasing concentration of serum triglycerides. This is in accordance to some other studies (Causey, Feirtag, Gallaher, Tunland, & Slavin, 2000; Letexier, Diraison, & Beylot, 2003). However, this effect did not pass the recommended threshold values that could negatively affect the health of investigated volunteers. Concerning glucose metabolism, the beneficial effects of prebiotics are linked to increased viscosity of the meal bolus, which delays and/or reduces carbohydrates absorption (Battilana et al., 2001; Wood et al., 1994). The increase in intestinal viscosity is dependent on the prebiotics molecular weight and solubility, on food processing, concentration and associated food matrix. Prebiotics enrichment of food

products has been largely studied, because it permits a higher intake per serving with a minimum decrease in palatability (Jenkins et al., 2002).

The results of anthropometric and body composition measurements, blood pressure and pulse are given in Table 6. No other statistically significant differences in the group of volunteers were observed after diet supplementation by the beverage.

## Conclusions

Calcium phosphate and calcium lactate together with vitamin D3 and prebiotic dietary fiber were used to fortify whey-based beverages. Both preparation of calcium showed non-significant impact for the human vision on color characteristics of the beverages. The changes in the taste and texture attributes of the beverages with the addition of calcium phosphate compared with calcium lactate showed a more significant decrease of their acceptability during storage. A comparison of the beverages with calcium phosphate and calcium lactate showed calcium lactate as more suitable for the tested food matrix and this formulation was selected for further testing.

The volunteers' blood analysis showed a significant decrease in LDL-cholesterol ( $P < 0.01$ ) and triglyceride ( $P < 0.01$ ) concentrations after a 21-day period consumption of drink with calcium lactate, vitamin D3 and prebiotic dietary fiber. According to the findings, these changes are likely to have a beneficial impact on health status of volunteers.

## Disclosure statement

The authors declare that there is no conflict of interest.

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Table 6. Anthropometric, body composition measurements, blood pressure and pulse data in the group of volunteers ( $n = 30$ ) before and after beverage supplementation.

Tabla 6. Medidas antropométricas, medición de la composición corporal, presión arterial y mediciones del pulso del grupo de voluntarios ( $n = 30$ ) antes y después del suplemento de bebida.

Parameter	Before	After	<i>t</i>	<i>P</i>
Weight (kg)	69.21 $\pm$ 10.18	69.24 $\pm$ 9.92	-0.178	0.860
Height (cm)	175.53 $\pm$ 6.81	175.54 $\pm$ 6.80	-1.000	0.327
Body mass index (kg/m <sup>2</sup> )	22.36 $\pm$ 2.09	22.37 $\pm$ 2.01	-0.232	0.819
Body fat (%)	26.46 $\pm$ 7.74	25.89 $\pm$ 7.84	0.681	0.502
Skeletal muscle (%)	32.86 $\pm$ 6.24	32.28 $\pm$ 7.08	0.990	0.332
Visceral fat (%)	3.76 $\pm$ 1.45	3.76 $\pm$ 1.59	0.000	1.000
Systolic blood pressure (mmHg)	133.36 $\pm$ 13.36	130.12 $\pm$ 13.62	1.431	0.165
Diastolic blood pressure (mmHg)	78.08 $\pm$ 7.27	75.16 $\pm$ 8.73	1.583	0.127
Pulse (rate/min)	83.08 $\pm$ 12.48	81.36 $\pm$ 13.29	0.700	0.490

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