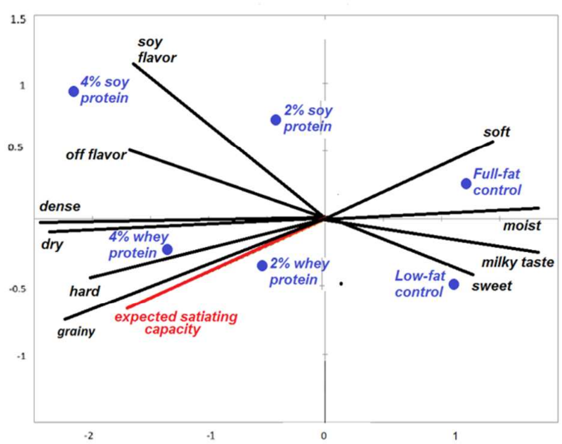
Food & Function

Accepted Manuscript



Increasing protein content proved to be a good strategy for enhancing expectations on satiating capacity of a cheese pie model.

Relating the effects of protein type and content in increased-protein cheese pies to consumers' perception of satiating capacity

J. Marcano[1], P. Varela[2] and S. Fiszman[1]

[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Agustín Escardino, 7, 46980 Paterna (Valencia), Spain

Nofima AS, P.O. Box 210, 1431 Ås, Norway

Abstract

Since protein has been shown to have the highest satiating-inducing effects of all the macronutrients, increasing the protein level is one of the main strategies for designing food with enhanced satiating capacity. However, few studies analyze the effect that protein addition has on the texture and flavor characteristics of the target food item to relate them to the expected satiating capacity it elicits. The present work studied cheese pies with three levels of soy and whey proteins. Since the protein level altered the rheological behavior of the batters before baking and the texture of the baked pies, the feasibility of adding several protein levels for obtaining a range of final products was investigated. A Check-all-that-apply question containing 32 sensory and non-sensory characteristics of the samples were performed with consumers (n=131) who also scored the perceived samples' satiating capacity. The results showed that the type and content of protein contributed distinctive sensory characteristics to the samples that could be related to their satiating capacity perception. Harder and drier samples (high protein levels) were perceived as more satiating with less perceptible sweet and milky cheese pie characteristic flavors Soy contributed off-flavour. These results will contribute a better understanding of the interrelation of all these factors, aiding the development of highly palatable solid foods with enhanced satiating capacities.

Introduction

The high prevalence of obesity in the industrialized world is partly due to the available food supply;[1] nowadays, the obesogenic environment, among other factors, triggers opportunities to unhealthy snacking in the western world, particularly among adolescents.[2] A useful approach for helping people to manage their appetite and food intake over the short term would be designing healthy everyday food products with enhanced satiating properties.[3] Three interrelated routes for food satiating enhancement.[4] The first route is to change the food composition to develop stronger physiological satiation and satiety signals, being satiety the process that causes one to stop eating, and satiation the feeling of fullness that persists after eating suppressing further food intake until hunger returns. The second route is to anticipate and build on smart external stimuli at the moment of purchase and consumption. Finally, the third route is to improve the palatability and acceptance of satiating capacity-enhanced foods. Based on these statements the present study will attempt the development of healthy snacks with enhanced satiating capacity.

The well-known satiating effect induced by protein-enriched meals in animals and humans has long been used in diets to decrease their sensation of hunger and lose weight.[5] In consequence increasing the amount of protein to reformulate a dairy protein-based food product was considered an interesting starting point in the present study. Proteins are widely cited in the literature as the macronutrient with the most potent satiety-inducing effect. The effects of high or normal casein-, soy-, or whey-protein breakfasts on specific hormones, amino acid responses and subsequent energy intake have been compared and found to be dose-dependent.[6] For example, gastrointestinally-digested 7S fraction of soy protein has significant CCK1R activity.[7] Contradictory results were obtained by other authors[8] varying the protein source in a mixed meal did not affect food behavior in healthy humans, probably because coingestion of carbohydrate and fat with protein buffers the kinetics of the physiological mechanisms involved in postprandial satiety after a protein load. Proteins from different sources can be added to food formulations to increase their protein content, and hence their satiating capacity; for this reason, two kinds of proteins: milk whey and soy, the most widely used in the industry, were selected for the present study. Increasing the amount of proteins will raise, in turn, the nutritional value of the final product. The latest research showed that protein quality evaluation in human nutrition should take into account their digestible amino acid contents, with each amino acid being treated as an individual nutrient; and the protein digestibility, considering the digestibility of the indispensable amino acids. Soy protein concentrate (SPC), compared with other vegetable sources, has good digestibility; however, differences in amino acid ileal digestibility were found between milk and soy for several amino acids as well as overall nitrogen digestibility, with the milk values being higher in all cases.

Whey protein isolated (WPI) consists mainly of β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins and lactoferrin[9] and glycomacropeptide corresponding to amino acid residues 106-169 from κ-casein. This macropeptide is released into the whey during the renneting of milk and is concentrated together with the whey proteins during the ultrafiltration of rennet whey.

In general, whey protein isolate (WPI) forms better heat-induced gels than whey protein concentrates and has better functional properties, which could be explained by the differences in composition, particularly a higher β-lactoglobulin content and lower fat, lactose and phospholipid contents, and by the extent of denaturation and protein aggregation. The low glycomacropeptide, non-protein-nitrogen and proteose peptone contents in WPI may also partly explain the superior gelation properties of these protein products.[10]

Soy protein concentrate (SPC) has a good aminoacid balance and is particularly rich in lysine. Soy proteins show health-promoting effects associated with reduced risk of cardiovascular disease, breast, prostate and colon cancers and bone health improvement.[11] SPC is typically prepared from milled soy-bean white flakes or flours by solubilizing the protein at pH 6.8–8 and 27–66ºC, using aqueous alkaline agents, followed by acidifying to pH 4.5 and concentrating the resulting curd by centrifugation after adjustment to pH 6.5–7.0 or by spray-drying the acidic form.[12]

Among other properties, as their gelling capacity, the water holding capacity (WHC) of proteins could perform an important role in the physical (e.g., elasticity, swelling), chemical (e.g., emulsification) and sensory (e.g., juiciness) attributes of food; in a comparison study among several proteins, soy protein had the highest WHC values, followed by whey protein[13]. Changes in rheology and texture could in turn affect the expected satiating capacity of protein-added products in terms of in-mouth residence time and the distinctive oral processing required. Therefore the effects of several contents of these two proteins on the batter rheology and final instrumental texture of the model food selected will be compared in the present study to check the feasibility of obtaining appropriate final products.

The importance of in-mouth cues, such as food texture, has been shown to play a role in eliciting satiating effects. In working with flavored milk drinks it was shown[14] that small changes in the sensory characteristics – thicker, creamier – changed the degree to which the beverages were perceived as being more satiating, that said, when predicted by relevant sensory cues. For this reason, when increasing protein contents to design foods with high satiating capacity, it is important to bear in mind that they bring about not only physical but sensory changes in the matrix of the reformulated food. This can influence how the consumers perceive the characteristics they associate with satiating capacity and thus change in their hedonic response.

Frankfurter-style sausages with double the protein of the normal formulation were perceived as more satiating during the first 90 min after the first meal and as less juicy, adhesive, harder and more granular than ones that contained less protein.[15] Astringency and the appearance of unpleasant notes such as “animal” or “musty” have been reported in high-protein satiating beverages formulated with whey proteins.[16] Some studies have suggested that proteins may be differentiated in terms of their satiating capacities. In the present study both distinctive sensory properties and expected satiating capacity provided by each protein to the model food will be evaluated.

A few examples of milk-based desserts with enhanced satiating capacity are already on the market, such as yogurts prepared with higher protein content than “normal”, and they are considered as a healthy snack. However, literature on the development of real food items with enhanced satiating capacity is still scarce. The fresh cheese pie selected as the model in the present study is a refrigerated dairy dessert that is basically made of fresh cheese, eggs, sugar, milk, and starch. It differs from American cheesecake in not having a crust and having a soft, spongy, moist, gel-like texture which can be cut with a knife. Fresh cheese is made from pasteurized non-cultured cows' milk and is characterized by a creamy, firm texture with a mild milky flavor. This dessert therefore presents a good option for increasing the protein content with the objective of enhancing its satiating effect, although changes in the texture and flavor of the final product may be expected, as well as changes in the rheology of the batter before baking.

The present study aims to evaluate the effect of adding increasing amounts of whey protein isolate (WPI) and soy protein concentrate (SPC) on the perception of enhanced satiating capacity of reformulated cheese pies.

The rheology of the batters before baking and the instrumental texture of the baked pies with different protein source and contents were investigated to obtain a range of pies without distorting the essence of the products. To know the distinctive characteristics that both proteins and their levels confer to the pies and which of them are more related to consumers' perception of satiating capacity a Check All That Apply question was performed.

Materials and Methods

Ingredients

Eight pie samples were formulated (Table 1). The ingredients were: full-fat fresh cheese (starter-free, pasteurized, protein content 10.9 g/100g, moisture 72 g/100g and fat 14 g/100g as declared by the supplier, Hacendado, Spain), low-fat fresh cheese (protein 12.3 g/100g, moisture 83 g/100g and fat content 0.2 g/100g, Hacendado, Spain), pasteurized liquid whole egg (Ovocity, Valencia, Spain), sucrose (Acor, Valladolid, Spain), maize starch (Maizena®, Barcelona, Spain), skimmed milk powder (Central Lechera Asturiana, Siero, Spain), whey protein isolate (WPI, Best Protein®, 90 g/100g protein content, Barcelona, Spain) and soy protein concentrate (SPC, protein content 69 g/100 g, Solcon IP Brenntag Quimica, Massalfassar, Spain).

Two control samples were prepared: one was prepared with full-fat cheese (the full-fat control or CFF) and another was prepared with 50:50 full-fat:low-fat cheese (the low-fat control or CLF), since the pie with total replacement by low-fat cheese collapsed after baking. In the present study, maximum replacement of full-fat cheese by low-fat cheese was sought, since the health concerns associated with fat consumption have led to an increase in the demand for low-fat dairy products, especially when designing foods with enhanced satiating capacity. Preliminary tests were run to obtain samples containing added WPI and SPC with the minimum amount of full-fat cheese in their composition. Bearing this in mind, samples W1, W2, and W3 were formulated with increasing amounts of whey protein isolate (WPI) and a 50:50 ratio of full-fat:low-fat fresh cheeses, while samples S1, S2, and S3 were formulated with increasing amounts of soy protein concentrate (SPC) and a 30:70 ratio of full-fat:low-fat fresh cheeses (Table 1), since soy protein conferred better viscosity/foaming properties on the batter than whey proteins. In this study, whichever the protein content added, the proportions of the other ingredients (cheese/ egg/ sugar, water/ milk powder and starch) remained the same as in the control sample.

Sample preparation

Batter preparation. The batter was prepared in a mixer (Kenwood Major Classic, UK), at top speed (580 rpm). Firstly, the cheese was whisked for 1 min, then the egg and sugar were added separately and mixed in for 1 min more after each addition. The milk powder was dissolved in water and the starch dispersed in it. These were added to the mixture, which was beaten for a further 1 min. Lastly, when necessary, the protein was added and the final mixture was beaten for 16 min. A total of 20 min processing was used for all formulations.

Baking. The batter was poured into a heat-resistant silicone mold for five rounded pies of 7 cm in diameter and 3.5 cm in height, and baked for 25 min at 180ºC in an electric oven (De Dietrich, Basingstoke, UK), preheated for 15 min. The oven, the tray and the tray position in the oven were identical in each case. The pies were left to cool at room temperature for 1 h, then demolded and stored in refrigeration (4 ºC) for 24 h before the measurements were made.

Small-strain rheological measurements of the batters

A controlled stress rheometer (AR-G2, TA-Instruments, Crawley, UK) was used. The strain applied was selected to guarantee the existence of linear viscoelastic response according to preliminary stress sweeps performed in all samples. The batters were all kept at 25ºC for 30 min after batter preparation before performing the rheological tests. The samples were allowed to rest in the measurement cell for a 5 min equilibration time. A 40-mm diameter plate-plate sensor geometry with a serrated surface and a 1 mm gap was employed. Vaseline oil was applied to the exposed surfaces of the samples to prevent sample drying during testing.

To simulate the effect of baking on the pie batter, temperature sweeps were performed from 25ºC to 80ºC at a heating rate of 1 ºC/min. Preliminary stress sweeps were carried out at 25ºC and at 80ºC to select strain values that would guarantee the existence of linear viscoelastic response. The temperature sweep was stopped at the corresponding temperature and after a 10 min temperature-equilibration time, the stress sweep was performed at 1Hz. In addition, mechanical spectra in the linear region from 10 to 0.01 Hz at 25 ºC and at 80 ºC (after the equilibration time and temperature sweep) were also recorded in separate tests. The storage modulus (G') and the loss modulus (G'') were recorded. Each formulation was measured twice from different batches. The data were recorded over time, using the TA data analysis software provided by the instrument's manufacturer.

Baked cheese pie instrumental texture measurements

The instrumental texture measurements were made with a TA.XT.plus Texture Analyser (Stable Microsystems, Godalming, UK). Each formulation was prepared twice, on different days. Three cylindrical pieces were cut from each baked pie with a round biscuit cutter. The top and bottom of each cylinder were discarded, leaving cylindrical pieces 22 mm in diameter and 17 mm in height. A double compression test (texture profile analysis, TPA) was performed at 8ºC with a flat-ended cylindrical probe (P/75), to reach 40% compression (6.8 mm of probe traveling distance) at a speed of 1 mm/s, with a 5 s waiting time between the two cycles. The parameters obtained from the curves were hardness (in N), springiness, cohesiveness, chewiness (in N) and resilience, using the Texture Expert software (version 6,0,6,0).

Consumer tests

Six samples were evaluated: CFF, CLF, W1, W3, S1, and S3 (Table 1). Samples with intermediate protein content (W2 and S2) were not evaluated to avoid consumers' fatigue, especially since the "sensation of fullness" will be scored.

All experiments were performed in compliance with the national legislation, and according to the institutional framework and practices established by CSIC Ethics Committee.

Check-all-that-apply (CATA) question

The CATA question, in which consumers describe a product by selecting appropriate words from a given list, has the advantage of obtaining direct consumer feedback, which is essential for reformulating existing products, among other applications.[17] Consumers (n=131) were recruited based on their consumption of the target product, as well as their interest and availability to participate; 65% were female, with ages ranging between 18 and 60 years. The test was performed in tasting rooms at the Institute of Agrochemistry and Food Technology (IATA-CSIC) and the Polytechnic University of Valencia. The consumer sample comprised varying household compositions, income levels, education levels, etc. but was not representative of general population in Valencia, Spain.

In the present study, 32 terms were selected from the available literature on sensory attributes of dairy desserts and on products with soy and whey protein added, and from informal tasting by researchers and a sensory descriptive panel. Some of these terms related to product use and satiating capacity-related sensations were also elicited for each pie. The instruction given to the participants was: "Please check all the answers that apply to the cheese pie you are tasting". Two groups of terms were included in the CATA question: sensory and non-sensory terms. The first group was composed of the following 28 sensory attributes: Hard, Spongy, Soy taste, Compact, Grainy, Moist, Cheese taste, Light texture, Floury taste, Sticky, Off-flavor, Fondant, Sandy, Tender, Dense, Gummy, Caramel taste, Creamy texture, Smooth, Sweet, Astringent, Earthy taste, Soft, Milky taste, Pasty, Dry, Porous texture, Starchy taste. The second group of 4 terms were usage and satiating capacity-related terms: "If I eat the whole pie I will not be hungry for a long time", that indicated high satiating capacity, “Even if I eat the whole pie I will be hungry shortly” that indicated low satiating capacity, “I would eat it as a snack” and “I would eat it as a dessert”. These terms were randomized within the two groups between products and across consumers. The test was recorded on paper and self-completed after instructions were given.

The pies were cut into eight wedge shaped pieces; the samples were assessed in a standardised tasting room equipped with individual booths.[18] Each consumer received the six samples of cheese pie in a sequential monadic series in a single session, following a balanced complete block experimental design (William’s design); he/ she was asked to indicate the CATA terms that applied to the samples which were described as “cheese pie samples”. Each sample was served in a small plastic tray coded with three digit random numbers; they were served at 10ºC. Still mineral water was available to rinse the mouth between samples; consumers were asked to rest for 30 seconds between samples.

Expected satiating capacity evaluation

Academia distinguishes between satiation and satiety; however, normal consumers are not aware of such difference.[19] In the present study after tasting the samples the consumers had to score their expectation on how filling each cheese pie is likely to be. In this scenario both concepts – satiety and satiation – are probably blurred. This reason is why the term “expected satiating capacity” was used in the results’ discussion.

After completing the CATA question the consumers scored the expected satiating capacity of each cheese pie sample on a nine-point scale, from 1=”If I ate this whole pie it would not fill me at all ” to 9=”If I ate this whole pie it would fill me a lot”. As the cheese pies were cut in eight wedge-shape pieces, the consumers could easily imagine the size of the whole cheese pie.

Data analysis

All the instrumental tests were carried out in duplicate with samples prepared on different days. Analyses of variance (ANOVA) were performed to compare the effect of adding the different proteins on the rheological and texture parameters of the cheese pie samples. When a significant difference ($p<0.05$) was detected in some variable, Tukey's means test was applied ($\alpha = 0.05$). The statistical analyses were performed with Statgraphics Centurion XVI (Warrenton, Virginia, USA).

The CATA results were analyzed for significant differences using non parametric tests. A chi square test was used to study the global differences between cheese pie samples in the CATA responses. Cochran's Q test[20] was performed to identify significant differences between samples for each of the terms included in the CATA question. For each cheese pie, the frequency of use of each sensory or usage attribute was determined by counting the number of consumers that selected that term to describe the corresponding sample.

A multiple factor analysis (MFA) was run on the CATA frequency counts of the significant attributes to understand the comparative positioning in sample and attribute two-dimensional plots as perceived by the consumers. Variables for the MFA were grouped as: taste, texture & non-sensory, with the intention to weight any possible effect, such as the final perceptual map is not dominated by only a few attributes.[21] Satiating capacity was used as a supplementary variable in the CATA data analysis to better understand its relation to the perceptual space. The statistical analyses were performed with XLStat 2010 software (Addinsoft, Paris, France).

Results and discussion

The aim of the present study was not to discover or fully characterize the structures that govern the mechanical behavior of the selected food systems, but to investigate the feasibility of obtaining a range of suitable cheese pies with increased amounts of proteins; for this reason the rheological behavior of the batters, their performance during baking and the final instrumental texture was studied. The added-protein pies obtained would show a series of sensory characteristics that would be potentially related to the satiating capacity expectations they elicited.

Small-strain rheological measurements of the batters

The batters obtained before cooking were subjected to rheological measurements to ascertain the effect of sample composition on the batter's viscoelasticity. The rheological features of a batter can give a good indication of its structure and, in consequence, of its final performance after baking.

The mechanical spectra of all the batter samples revealed the existence of soft gels, with higher G' values than G'' values and strong frequency dependence throughout the frequency range measured. Fig. 1a shows the mechanical spectra of samples CFF, CLF, W3 and S3. The mechanical spectra of the samples with lower whey protein (W1 and W2) and soy protein (S1and S2) levels showed similar patterns to samples W3 and S3 respectively, but with lower viscoelastic values.

Comparison of samples CFF and CLF (without added protein) showed that the full-fat cheese sample behaved as a weaker gel. The two reasons that could underlie this result are, firstly, that the low-fat cheese had a higher protein content than the full-fat cheese (see the ingredient content in Table 1), so it would form a stronger protein network, and secondly, that the full-fat sample (CFF) contained a higher number of fat globules, which deform easily, producing a more deformable matrix.

Adding WPI (sample W3) caused an evident increase in viscoelastic behavior compared to the control sample (CFF) (Table 2). As previously stated[22] the presence of whey protein in fat-free dairy desserts promotes the formation of stronger gel structure as a result of protein-protein interactions. The functional behavior of milk whey proteins is very complex, caused by interactions between the proteins' intrinsic properties such as composition and amino acid sequence, molecular weight, conformation, flexibility, net charge and hydrophobicity, and extrinsic factors such as temperature, pH and other food components. It was found that adding WPI to low-fat cheese favored the formation of a close, compact protein network.[23]

In contrast, the added-soy protein sample (S3) contained a very similar protein level to W3 but showed weaker viscoelastic behavior, similar to that of the control sample (CFF) (Table 2). A thickening effect of SPC was visible during batter mixing in the preliminary formulation trials, which allowed higher amounts of full-fat cheese to be replaced in the formulation without collapsing the final pie structure. Several studies have demonstrated that heat treatment strongly influences the protein–protein interaction of soy proteins, and also their functional properties, such as gelation.[24] Heat denaturation has been considered the main factor in soy gel formation and the type of gel depends on the heating and cooling conditions, among other factors.

The evolution of the viscoelastic properties as the temperature increased (Fig. 1b) made it possible to predict the behavior of the batters during baking by showing the structural changes that took place. Sample S3 showed the earliest increase in viscoelastic properties with heating, acquiring a strong structure (higher viscoelastic parameter values) at lower temperatures than the other samples. The rest of the batters behaved in a very similar way: a gradual increase in the viscoelastic parameters took place over the heating period due to gelatinization of all the proteins in the batters (egg, milk, and added whey) and at approximately 75ºC a steeper increase in the values indicated the gelatinization of the starch.

Both the mechanical spectra and the thermal behavior of the batters showed the feasibility of using both protein types in the selected amounts to reformulate cheese pies with enhancing satiating capacity.

Cheese pie instrumental texture measurements

A series of composition factors could influence the final texture of the cheese pie models. The complex variety of ingredients makes it difficult to know exactly which one determines any feature of their final texture. Rather, this is the result of the combination of them all. Besides the starch in the samples, which is probably responsible for part of the structure after reaching its gelatinization temperature, a range of proteins (egg, milk, cheese and added soy or whey protein) are probably the most important factor in determining the final texture of the pies, again due to their denaturation and subsequent gelation. Understanding the properties of specific proteins and ingredients is very useful but is restrictive in predicting performance in real foods[25], where the complexities of ingredients and processing operations have a significant effect on the colloidal structures and therefore on the overall properties of the final food product. One of the most relevant properties of proteins in food systems is their ability to form gels after heating. Heat gelation contributes to textural properties, shapes the product, holds other food components together and retains water in the product. Gelling involves hydrophobic interactions, electrostatic and disulfide bonds. Gelling properties and other functional properties of protein isolates and concentrates are influenced by the physicochemical properties of the proteins, which change as a function of process variables, such as protein concentration, heating temperature and time, ionic strength, and pH.[26]

Fig. 2 shows the behavior of the samples during instrumental texture profile analysis (TPA). The hardness and chewiness parameters (Table 3) clearly show that increasing concentrations of WPI and SPC in the samples produced statistically significant higher values ($p<0.05$) compared with the CFF and CLF samples, with the added-whey protein samples being significantly harder than the added-soy protein samples. In turn, the mean TPA hardness and chewiness values grew significantly higher in the following order: W3 > W2 > W1 and S3 > S2 > S1. The replacement of 50% of full-fat cheese with low-fat cheese (sample CLF) also produced a significant increase in hardness compared to CFF. These results related well to the rheological results for the corresponding batters before cooking, which showed the W3 samples as having the strongest viscoelastic properties, and sample S3 behavior as nearer to that of sample CFF.

In a number of previous studies, different whey proteins' addition has produced harder food matrices. For example, processed cheese analogues prepared with whey protein concentrate (WPC) were found to be harder than those prepared with other proteins,[27] while cheeses to which WPC had been added in connection with partial or total removal of fat had a more compact matrix structure.[19] In the present study, the mean cohesiveness and springiness values did not differ significantly between samples (Table 3). These results indicated that a range of pies with different instrumental textures were obtained by adding whey and soy proteins at different levels without distorting the essential nature of the product.

Consumer tests

Check-All-That-Apply (CATA) question

Although traditional product characterization techniques such as Quantitative Descriptive Analysis provide accurate and reliable information, consumer sensory product characterization methods are interesting options when studying consumer perception[28]. CATA question constitutes a simple and valid approach that is increasingly being used to capture consumer information about sensory and non-sensory perceptions of food products. The present case constitutes a new application of this method to know how the addition of proteins affects a novel, reformulated food product to obtain added value: enhanced satiating capacity along with better nutritional value. This method has the advantage of gathering information on perceived product attributes without requiring scaling, allowing for a slightly less contrived description of the main sensory properties of the product tested.

Table 4 shows the frequency with which each term in the CATA question was selected by the consumers to describe the cheese pies. Significant differences were found in the frequencies of 29 out of the 32 terms related to texture, flavor or use elicited by the samples, suggesting that this type of question was able to detect differences in the consumers' perceptions of the cheese pie samples. No significant differences between samples ($p<0.05$) were found for "Pasty", "Sticky" and "Even if I eat the whole pie I will be hungry shortly", which could probably be due to these attributes were equally relevant for all the samples; also, those three terms had generally a low number of selections (less than 24). There were other attributes also selected by a low number of consumers: "Caramel taste", "Gummy", "Porous texture", "Astringent" and "Soy taste".

With regard to texture terms, the CFF and CLF samples were mostly described by the attributes "Soft", "Creamy", "Tender", "Light", "Moist", "Fondant" and "Spongy". In contrast, samples W1, W3, S1 and S3 were described using words such as "Hard" (W3, S3), "Compact" and "Dry" (both particularly high for W3), "Dense", "Floury" and "Sandy", which reflected opposite texture characteristics. These results were in line with the instrumental texture assessment of the samples, which clearly showed that the added-soy and -whey protein samples were harder and more chewy. The rheological results that indicated a more solid structure of the whey protein samples and early heat response of the soy protein would indicate that a more solid network was formed by increased-protein samples.

With respect to terms of taste, CFF and CLF samples were mostly described by the attributes "Sweet", "Cheese taste" and "Milky taste". These flavor terms were also selected to describe the added-protein samples W1, W3 and S1, but less frequently than for samples CFF and CLF. In contrast, the frequency of use of the terms “Starchy taste" "Earthy taste" although not very high, increased in the added-protein samples, especially in S3, which showed significantly higher frequency of these than the rest of the samples, and also of "Soy taste" and "Astringent". Also, "Off flavor" was significantly higher for S3, selected by 30% of consumers. This increased frequency of selection of negative attributes would probably indicate that soy protein introduces flavor notes which were perceived as striking or out of place in a cheese pie.

Regarding the CATA terms related to the perception of satiating capacity, the consumers selected "If I eat the whole pie I will not be hungry for a long time" more frequently than "Even if I eat the whole pie I will be hungry shortly" and an increase in its frequency was observed in the samples with increased protein concentrations. The consumers selected "I would eat it as a dessert" more frequently than "I would eat it as a snack", indicating that the samples were considered more as complementing a meal than as a snack to be eaten between meals or a meal replacement. This result could be also influenced by the fact that “normal” cheese pie (the base formulation, CFF) is eaten as a dessert and the consumers who completed the CATA question were not aware of the aim of the study or of the sample formulations. Sample S3 had low frequency values for these two terms. In this case, the higher frequency of attributes such as “Off-flavor”, “Starchy taste”, “Soy taste”, and “Floury taste” than for any of the other samples could contribute to consumers’ not thinking it could be eaten either as a dessert or as a snack.

The first two factors of the MFA (Fig. 3) accounted for 92.72 % of the variance of the original dataset, representing 77.65% and 15.08% of the variance respectively. Most of the CATA terms were well represented in the perceptual space defined by the first two factors of the MFA. The first factor (X axis) was related to a series of texture terms such as “soft,” “moist”, or “light”. “Creamy”, “tender” and “fondant” were placed on the right of this axis (positive values) and the evidently contrasting terms “dense”, “compact,” “dry,” “gummy” and “hard” on the left side (negative values). Regarding flavor, the first dimension of the MFA also contrasted “milk taste”, “caramel taste” and “sweet” (positive values) with the terms “astringent”, “earthy taste”, “off-flavor” and “floury taste” (negative values). The second factor of the MFA was mainly correlated to “soy taste” and "starchy taste" (positive values of the Y axis), opposite to "cheese taste" (negative values of the second component).

The relationship between in vivo aroma release and perception in food products is strongly dependent on the type of texture, relative to two mechanisms: physicochemical mechanisms based on the modification of aroma release and a cognitive mechanism based on aroma-taste-texture interactions. These two mechanisms exist simultaneously but have more or less impact on aroma perception depending on the type of texture.[29]

Finally, the terms related with uses such as "I would eat it as a snack" and "I would eat it as a dessert" were placed in the right side of the X-axis, opposite "If I eat the whole pie I will not be hungry for a long time".

The products were well differentiated on the first two dimensions. Samples CFF and CLF were close to each other in the area defined by soft, tender samples with a sweet, milky taste. These two samples were the only ones placed on the right side of the sample plot. All the added-protein samples appeared on the left side and were grouped by protein type, in both cases with the more concentrated ones to the left of the least concentrated ones. This indicates that the higher the protein concentration the harder, drier, and more compact the texture. The area defined by the soy-protein samples (S1 and S3) coincided with the flavours not desirable in a milk-based product (upper side), whereas the milk-protein samples (W1 and W3) were placed further away from these flavour features.

Expected satiating capacity assessment

Sample W3 was the only one that elicited a significantly higher expectation of satiating capacity (Table 4). In order to relate the values obtained to the texture and flavour drivers, expected satiating capacity was mapped as a supplementary variable on the MFA (Fig. 3). This means that it was not taken into account during the construction of the factorial axes while the others were considered active, but the statistics for this supplementary variable were obtained by projecting this element onto the active space. The expected satiating capacity position was well in the direction of samples W1 and W3, indicating that the hard, grainy, sandy, dry texture of these samples elicited higher satiating expectations than the lighter texture of the samples without added protein. In particular, when looking at the individual frequencies of mention for the attributes on table 4, W3 was particularly selected as harder, dryer and more compact than the rest of the samples.

The texture features contributed by the addition of protein implied more labour-intensive oral exposure and processing. Previous studies[1] have suggested that particular oral processing characteristics such as a lack of chewing activity contribute to low satiating efficiency of foods. On the other hand, for equal calories, oral-sensory exposure time could contribute to higher satiation within a meal by triggering anticipatory responses. This is because animals, including humans, learn to associate the sensory characteristics of a food with its caloric value post-consumption.[30] These associations are likely to influence explicit expectations about the effect a food will have on appetite, including how filling a food is likely to be (expected satiation) and the extent to which it will stave off hunger until the next meal (expected satiety). Such expectations have been shown to influence appetitive satisfaction and portion size selection.[31] In particular, a previous study[32] found that the expected satiation of semi-solid dairy products increased consistently with increasing thickness. In an extensive review paper on texture and satiation[33], it was concluded that longer sensory exposure times lead to cephalic phase responses that not only contribute to physiological homeostasis but also contribute significantly to satiety.

Conclusions

The satiating effect of protein is recognized worldwide. Consequently, formulating food with the addition of extra protein would seem to be a logical way to enhance the satiating capacity of foods. However, this addition modifies the texture and flavor characteristics of the final products, which in turn influences expectations of satiating capacity. In the present study, a cheese pie was selected as a model food for incorporating different levels of soy and milk whey proteins with the aim of obtaining a range of feasible enhanced satiating capacity products. The results show that the added proteins make the texture harder and drier and move the food away from the sweet and milky flavors that are well-known to consumers. Importantly, these texture characteristics elicited stronger expected satiating capacity, probably due to the longer and more laborious chewing or oral processing needed in order to swallow these samples comfortably. The fact that the addition of whey showed to have an enhanced perception of satiating capacity, without the off-flavors provided by the soy proteins, suggest that whey proteins could be better candidates for reformulation.

The present work opens the door to new strategies for achieving food items with enhanced expectations on satiating capacity, bearing in mind that it is important to deliver matching expectations in the consumers at a very early stage of consumption, as these are drivers of early satiation.

Acknowledgements

The authors are grateful to the Spanish Ministry of Science and Innovation for financial support (AGL2012-36753-C02-01). They would also like to thank Mary Georgina Hardinge for assistance in translating and correcting the English manuscript.

References

1. C. G. Forde, N. van Kuijk, T. Thaler, C. de Graaf and N. Martin, Oral processing characteristics of solid savoury meal components, and relationship with food composition, sensory attributes and expected satiation, *Appetite,* 2013, **60**, 208–219.
2. S. Fiszman and P. Varela, The role of gums in satiety/satiation. A review, *Food Hydrocolloid*, 2013, **32**, 147-154.
3. E. Almiron-Roig, D. Grathwohl, H. Green and A. Erkner, Impact of some isoenergetic snacks on satiety and next meal intake in healthy adults, *J. Hum. Nutr. Diet.*, 2009, **22**, 469-474.
4. E. Van Kleef, J. Van Trijp, J. Van Den Borne and C. Zondervan, Successful development of satiety enhancing food products: towards a multidisciplinary agenda of research challenges, *Crit. Rev. Food Sci. Nutr.*, 2012, **52**, 611-628.
5. A. Penhoat, E. Mutel, M. Amigo-Correig, B. Pillot, A. Stefanutti, F. Rajas and G. Mithieux, Protein-induced satiety is abolished in the absence of intestinal gluconeogenesis, *Physiol. Behav.*, 2011, **105**, 89–93.
6. M. Veldhorst, A. Nieuwenhuizen, A. Hochstenbach-Waelen, A. Van Vught, K. Westerterp, M. Engelen, R. Brummer, N. Deutz and M. Westerterp-

Plantenga, Protein-induced satiety: Effects and mechanisms of different proteins, *Physiol. Behav*, 2009, **96**, 675-682.

7. D. Staljanssens, J. Van Camp, A. Billiet, T. De Meyer, N. Al Shukor, W. De Vos and G. Smagghe, Screening of soy and milk protein hydrolysates for their ability to activate the CCK1 receptor, *Peptides*, 2012, **34**, 226–231
8. V. Lang, F. Bellisle, J. Oppert, C. Craplet, F. Bornet, G. Slama and B. Guy-Grand, Satiating effect of proteins in healthy subjects: a comparison of egg albumin, casein, gelatin, soy protein, pea protein, and wheat gluten, *Am. J. Clin. Nutr.,* 1998, **67**, 1197–1204.
9. D. Zhu, S. Damodaran, and J. Lucey, The formation of WPI-dextran conjugates in aqueous solutions, *J. Agr. Food Chem.*, 2008, **56**, 7113-7118.
10. P. Lorenzen and K. Schrader, A comparative study of the gelation properties of whey protein concentrate and whey protein isolate, *Lait*, 2006, **86**, 259-271.
11. M. Majzoobi, F. Ghiasi, M. Habibi, S. Hedayati and A. Farahnaky, Influence of Soy Protein Isolate on the Quality of Batter and Sponge Cake, J. Food Process. Pres., **38**, 1164-1170.
12. E. W. Lusas and M. N. Riaz, Soy protein products: processing and use, *J. Nutr*., 1995, **125**, 573-580.
13. P. Wong and D. Kitts, A Comparison of the Buttermilk Solids Functional Properties to Nonfat Dried Milk, Soy Protein Isolate, Dried Egg White, and Egg Yolk Powders, *J. Dairy Sci*., 2003, **86**, 746-754.
14. M. Yeomans and L. Chambers, Satiety-relevant sensory qualities enhance the satiating effects of mixed carbohydrate-protein preloads, *Br. J. Nutr.*, 2011, **111**, 1137-1144.
15. H. K. Sivertsen, Ø. Ueland and F. Westad, Development of satiating and palatable high-protein meat products by using experimental design in food technology, *Food Nutr. Res*., 2010, **54**, 5114.
16. N. Little, S. Gregory and N. Robinson, Protein for satiety. The direction is clear, *Agro Food Ind.Hi-Tech*, 2009, **20**, 23-25.
17. G. Ares, C. Barreiro, R. Deliza, A. Giménez and A. Gámbaro, Application of a check-all-that-apply question to the development of chocolate milk desserts, *J. Sens. Stud*., 2010, **25**, 67-86.
18. ISO. Sensory analysis General guidance for the design of test room.

Standard No 8589, Geneva, Switzerland: International Organization for Standardization, 2007.

19. S. Fiszman, P. Varela, P. Díaz, M. B. Linares and M. D. Garrido, What is satiating? Consumer perceptions of satiating foods and expected satiety of protein-based meals, *Food Res. Int.*, 2014, **62**, 551–560.
20. E. Manoukian. Mathematical nonparametric statistics. Gordon & Breach, 1986.
21. M. Meyners, J. C. Castura and B. T. Carr, Existing and new approaches for the analysis of CATA data *Food Qual. Pref.*, 2013, **30,** 309–319.
22. M. Vidigal, V. Minim, A. Ramos, E. Ceresino, M. Diniz, G. Camilloto and L. Minim, Effect of whey protein concentrate on texture of fat-free desserts: sensory and instrumental measurements, *Ciênc. Tecnol. Aliment*, 2012, **32**, 412-418.
23. C. Lobato-Calleros, J. Reyes-Hernández, C. Beristain, Y. Hornelas-Uribe, J. Sánchez-García and E. J. Vernon-Carter, Microstructure and texture of white fresh cheese made with canola oil and whey protein concentrate in partial or total replacement of milk fat. *Food Res. Int.*, 2007, **40**, 529–537.
24 R. Roesch and M. Corredig, Texture and microstructure of emulsions prepared with soy proteins concentrate by high-pressure homogenization, *LWT-Food Sci. Technol*., 2003, **36**, 113–124.
25. E. Foegeding and J. Davis, Food protein functionality: A comprehensive approach, *Food Hydrocolloid*, 2011, **25**, 1853-1864.
26. Hua, S. Cui, Q. Wang, Y. Mine and V. Poysa, Heat induced gelling properties of soy protein isolates prepared from different defatted soybean flours, *Food Res. Int.*, 2005, **38**. 377-385.
27. B. Sołowiej, I. Cheung and E. Li-Chan, Texture, rheology and meltability of processed cheese analogues prepared using rennet or acid casein with or without added whey proteins, *Int. Dairy J.*, 2014, **37**, 87-94.
28. P. Varela and G. Ares, Sensory profiling, the blurred line between sensory and consumer science. A review of novel methods for product characterization, *Food Res. Int*., 2012, **48**, 893–908.
29. I. Gierczynski, E. Guichard and H. Laboure, Aroma perception in dairy products: the roles of texture, aroma release and consumer physiology. A review, *Flavour Frag. J.*, 2011, **26**, 141-152.

30. M R. Yeomans, L. Weinberg, and S. James, Effects of palatability and learned satiety on energy density influences on breakfast intake in humans, *Physiol Behav.,* 2005, **86,** 487–499.
31. K. McCrickerd, L. Chambers, J. M. Brunstrom, J. E. Norton, T. Mills and M. R. Yeomans, Subtle changes in the flavour and texture of a drink enhance expectations of satiety, *Flavour*, 2012, 1:20. Retrieved from: http://www.flavourjournal.com/content/1/1/20.
32. P. Hogenkamp, A. Stafleu, M. Mars, J. Brunstrom and C. De Graaf, Texture, not flavor, determines expected satiation of dairy products, *Appetite*, 2011, **57**, 635–641.
33. C. De Graaf, Texture and satiation: the role of oro-sensory exposure time, *Physiol. Behav.*, 2012, **107**, 496–501.

Table 1. Composition of the cheese pie samples.

Ingredient (g)	Sample							
	CFF	**CLF**	**W1**	**W2**	**W3**	**S1**	**S2**	**S3**
Fresh cheese	55.00	27.50	26.95	26.67	26.40	16.17	16.00	15.84
Low-fat fresh cheese	0	27.50	26.95	26.67	26.40	37.73	37.35	36.96
Whole egg	20.00	20.00	19.60	19.40	19.20	19.60	19.40	19.20
Sucrose	10.00	10.00	9.80	9.70	9.60	9.80	9.70	9.60
Water	8.00	8.00	7.84	7.76	7.68	7.84	7.76	7.68
Maize starch	5.00	5.00	4.90	4.85	4.80	4.90	4.85	4.80
Skimmed milk powder	2.00	2.00	1.96	1.94	1.92	1.96	1.94	1.92
WPI	0	0	2.00	3.00	4.00	0	0	0
SPC	0	0	0	0	0	2.00	3.00	4.00
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Total protein content	9.2	9.6	11.2	12	12.8	10.9	11.5	12.1
Total fat content	9.8	6.0	5.9	5.9	5.8	4.5	4.4	4.4

CFF: Control full-fat; CLF: Control low-fat; W1, W2 and W3: added whey protein (2, 3, and 4 g/ 100g respectively); S1, S2, and S3 added soy protein (2, 3, and 4 g/ 100g respectively

Table 2. Mean values (n=2) of G', G'' and tgδ at 1 Hz at 20ºC, 60ºC and 80ºC for cheese pie samples. For sample codes see Table 1

Sample	20ºC			60ºC			80ºC		
	G' (Pa)	G'' (Pa)	tgδ	G' (Pa)	G'' (Pa)	tgδ	G' (Pa)	G'' (Pa)	tgδ
CFF	9.0^{a}	4.0^{a}	0.48^{a}	29.2^{a}	13.6^{a}	0.47^{a}	189.8^{a}	59.2^{a}	0.32^{a}
CLF	16.6^{a}	5.8^{a}	0.46^{a}	39.0^{a}	17.9^{a}	0.46^{a}	208.3^{a}	62.5^{a}	0.22^{a}
W3	43.3^{a}	11.6^{a}	0.48^{a}	39.1^{a}	18.1^{a}	0.36^{a}	45.0^{a}	65.3^{a}	0.30^{a}
S3	10.2^{a}	5.5^{a}	0.55^{a}	62.1^{b}	28.9^{b}	0.47^{a}	324.0^{a}	108.1^{a}	0.34^{a}

Values with different superscript letters in the same column denote statistically significant differences ($p<0.05$)

Table 3. Mean values (n=2) of the texture profile analysis (TPA) parameters of the cheese pie samples

Sample	TPA parameters			
	Hardness (N)	Cohesiveness	Springiness	Chewiness (N)
CFF	2.97^{a}	0.44^{a}	0.70^{a}	0.92^{a}
CLF	3.61^{b}	0.45^{a}	0.68^{a}	1.12^{a}
S1	4.60^{c}	0.42^{a}	0.65^{a}	1.25^{ab}
S2	5.23^{d}	0.43^{a}	0.64^{a}	1.45^{b}
S3	5.78^{e}	0.45^{a}	0.70^{a}	1.81^{c}
W1	6.43^{f}	0.42^{a}	0.64^{a}	1.75^{c}
W2	8.32^{g}	0.44^{a}	0.68^{a}	2.51^{d}
W3	9.57^{h}	0.41^{a}	0.68^{a}	2.64^{d}

Different superscript letters in the same column denote values with statistically significant differences ($p<0.05$)

Table 4. Frequency of selection of CATA terms for the six cheese pies and Cochran's Q test for significant differences between them. Mean expected satiating capacity mean scores and their significant differences (Tukey's test).

CATA term	**Sample**						**Cochrane's Q**
	CFF	**CLF**	**W1**	**W3**	**S1**	**S3**	
Sensory terms							
Sweet	54	42	31	18	27	12	<0.0001
Smooth	53	47	16	13	27	5	<0.0001
Soft	47	52	14	6	32	5	<0.0001
Cheese taste	45	39	41	30	22	8	<0.0001
Milky taste	44	38	41	24	31	9	<0.0001
Creamy texture	40	41	11	4	22	3	<0.0001
Tender	39	38	18	10	17	4	<0.0001
Light	37	26	14	7	18	9	<0.0001
Moist	31	36	16	6	17	7	<0.0001
Fondant	28	28	4	8	16	5	<0.0001
Spongy	27	44	7	10	14	8	<0.0001
Grainy	27	16	40	48	25	45	<0.0001
Caramel taste	18	17	11	7	9	5	0.001
Sandy	17	10	29	40	24	34	<0.0001
Pasty*	15	17	24	21	21	25	0.277
Sticky*	15	8	11	16	17	8	0.060
Compact	12	12	39	59	31	43	<0.0001
Starchy taste	9	7	11	12	27	31	<0.0001
Floury	9	10	23	29	31	42	<0.0001
Gummy	8	9	18	22	14	18	0.005
Dry	6	8	32	59	26	52	<0.0001
Dense	6	12	31	34	26	37	<0.0001
Earthy taste	5	5	12	22	12	32	<0.0001
Porous texture	4	15	9	12	24	15	<0.0001
Off flavor	3	11	17	15	16	40	<0.0001
Astringent	1	2	5	6	4	15	<0.0001
Soy taste	0	6	3	5	9	13	<0.0001
Hard	0	4	17	40	10	31	<0.0001
Non-sensory terms							
I would eat it as a dessert	40	47	28	22	24	7	<0.0001
I would eat it as a snack	27	27	21	17	22	10	0.000
Even if I eat the whole pie I will be hungry shortly*	18	9	11	11	14	8	0.075
If I eat the whole pie I will not be hungry for a long time	15	22	24	29	23	21	0.031
Expected satiating capacity scores	5.6^{a}	5.4^{a}	5.8^{a}	6.3^{b}	5.6^{a}	5.9^{a}	

*Indicates a term with no significant differences (Cochran's Q test, p>0.05) among samples

$^{a.b}$ Different superscript letters denote significant differences (Tukey's test , p<0.05)

Figure captions

Figure 1. Rheological behavior of the batters without added protein (CFF and CLF) and with the highest protein content (W3 and S4) before cooking. a) Mechanical spectra; b) G' and G'' values as a function of increasing temperature. Heating rate:1ºC/min ; frequency: 1Hz

Figure 2. Texture profile analysis (TPA) of the cheese pies without added protein and with the three levels of soy and whey protein

Figure 3. a) Map of terms from the check all that apply (CATA) question; b) Representation of the cheese pies without protein addition and with the highest level of soy and whey protein in the first two dimensions of the Multiple Factor Analysis (MFA) of the CATA counts