A Study of Semi-Hard Plant-Based Imitation Cheese

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ABSTRACT

Plant-based cheese has increased in popularity. But often plant-based cheeses have low protein contents and a low likeability. This study aimed to produce a plant-based cheese analogue with functionality similar to a traditional Danish semi-hard cheese. Plant-based imitation cheese samples were produced in a Thermomix® and evaluated by texture analysis, and sensory descriptive analysis, and the microstructure was studied with Confocal Laser Scanning Microscopy. Results revealed that the combination of pea protein and chlorella protein increased textural stress compared to pea protein alone or a mix of pea and faba bean protein. Pea and pea-chlorella samples had stress values similar to the reference. The mix of pea and pea-faba bean differed from the remaining samples in microstructure and in the sensory profile. Overall, this study revealed that combining protein from different plant sources might be the best option to mimic certain food product behaviour.

INTRODUCTION

Plant-based cheese has increased in popularity over the past years. This is due to an increased focus on sustainability, the environment, and animal welfare[1]. The production of plant-based cheese is however challenging. Plant-based cheese has low likeability regarding flavour, texture, and value for money[1]. Another consideration in plant-based products is the low protein content. Often the protein content in plant-based cheese is as low as 0-4% in comparison to dairy cheese with up to 42% protein.

In plant-based cheese, the ingredients are of great importance for functionality. Mimicking dairy protein functionality with plant proteins is complex, as the plant proteins are different, both physically and chemically[2]. Typically, a significant part of the plant-based cheeses is contributed from starch. Some of the challenges when substituting dairy protein with plant proteins in cheese include reduced solubility, emulsification, and gelling properties[3]. Plant protein functionality is affected by many factors, such as source, cultivar, extraction method, and drying method. Studies have shown that a combination of plant proteins from different sources can be a method to improve functionality[3].

The project aimed to produce a plant-based cheese analogue with a texture similar to a dairy cheese. Furthermore, the aim was to include as much vegetable protein as possible, to produce a product with high nutritional quality.

MATERIALS AND METHODS

Materials

Plant-based imitation cheese samples were produced from pea protein (Roquette, Lestrem, France), pea-Faba protein mix (Plant Mate, Silkeborg, Denmark), chlorella algae (Aliga, Hjørring, Denmark), Potato starch (KMC), Rapeseed oil (Rema 1000, Denmark), aroma (Ingredients DSM, Heerlen, Netherlands) and demineralized water.

A reference Danbo 30+ (Rema 1000, Denmark) was included. The composition of the reference was 16% fat, 1.8% salt, 26% protein, and > 56% water /100g.

Sample production

Four different plant-based imitation cheese samples were produced in 300 g batches, composition in **Table 1**.

TABLE 1: Sample composition (%) and calculated protein content.

Ingredient	E1	E2	E3	E4
Pea	10.53	5.10	8.78	10.53
Pea-Faba bean mix	-	8.53	-	-
Chlorella	-	-	2.32	2.32
Starch	20.47	17.37	19.90	18.14
Oil	13.00	13.00	13.00	13.00
Water	54.85	54.85	54.84	55.36
Aroma	1.16	1.16	1.16	0.64
Calculated protein content (%)	9 % Pea	4.5 % Pea + 4.5 % mix	7.5% pea + 1.5 % Chlorella	9 % pea + 1.5 % Chlorella

Samples were produced in a Thermomix® TM6 (Vorwerk International Strecker & Co., Switzerland) by shearing and heating the ingredients. After production, samples were transferred to an aluminum foil container and cooled down to 5 ± 1 °C for 24 hours prior to analysis.

Texture analysis

Texture analysis was performed by uniaxial compression on samples and reference using a TA HDi Texture Analyzer (Stable Micro Systems, Godalming, UK). A compression speed of 0.8 mm $s^{-1}$was used and the deformation was measured until break. Texture analysis was performed at room temperature (20-22 °C), with a sample temperature of 10 ± 1 °C. Six repetitions were made for each sample.

Sensory analysis

A sensory descriptive analysis was performed over two weeks with a trained sensory panel consisting of eight assessors. Before the sensory analysis, the assessors developed the vocabulary and had a training session. Fresh samples were produced for every session. Samples were cut into cubes of 1.5 x 1.5 x 3 cm^3, equal to 10 - 15 g, and placed in ramekins marked with a random three-digit number. Assessors were asked to evaluate texture, aroma, flavour, mouthfeel, and aftertaste. This manuscript will only consider texture. Attributes of texture in the sensory descriptive analysis were: Elastic/Bouncy, Firmness, Fracturability, Chewiness and Fast dissolving. The attributes were evaluated on a 15 cm scale varying from “very low” to “very high” intensity. For each evaluation round three replicates were prepared, and samples were served in randomised order.

Confocal Laser Scanning Microscopy

Microscopy slides were prepared by addition of 30 µL Fluorescein isothiocyanate (FITC) (0.2 mg/mL in acetone), 30 µL Nile Red (0.1 mg/mL in acetone), and 20 µL Fast Green (0.1 mg/mL in water). Imaging was performed using a Nikon Ni-E upright confocal laser scanning microscope (Nikon Instrument Inc. Tokyo. Japan) using a 60 × objective. Laser lines of 488 nm, 561 nm, and 640 nm were used for excitation to induce fluorescence emission of the three dyes, respectively.

RESULTS AND DISCUSSION

Texture profile analysis

Results from stress measurements **Fig 1** was first used to evaluate the repetitions. Looking at the repetitions from day 2 and forward there were no significant differences. Day 1, however, differed from the remaining, due to a cooling mistake, since this batch was only cooled to 10 °C and not 5 °C, which affected the texture. Comparing the samples cooled to 5 °C indicated that the Thermomix® was a useful tool for the production of plant-based imitation cheese samples.

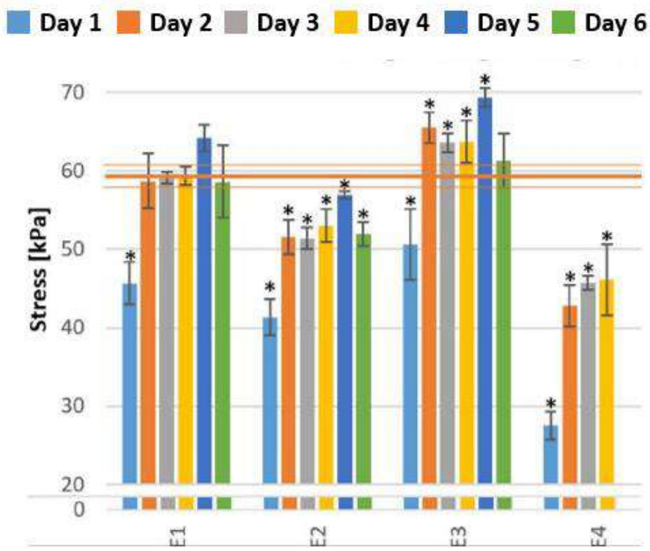

FIGURE 1: Texture analysis results on stress at breaking of plant-based cheese samples E1-E4 (see Table 1 for sample composition). The horizontal orange line is the stress value for a reference semi-hard dairy cheese. "*" indicates which samples are significantly different from the reference. Colour indicate batches.

E1, E2, and E3 all had higher stress values than E4. This could be related to the higher calculated total protein content of 10.5% in E4. When comparing E1, E2, and E3, all with a calculated protein content of 9%, E3 with chlorella protein and pea protein had the highest stress value followed by E1 with pea protein only. This indicated that the mix of chlorella protein and pea protein had an interaction effect leading to an increased stress value.

On the contrary, there was a negative effect of mixing pea protein with pea-faba bean protein mix, as here a lower stress value was observed. Comparing all samples to the reference dairy semi-hard cheese E1 was closest in stress value, whereas E2 and E4 were lower and E3 had a higher stress value.

Sensory texture

The texture stress measurement was followed by a sensory descriptive analysis with five attributes. The texture profiles of the four plant-based imitation cheese samples are shown in **Table 2**. ANOVA and a post hoc test revealed significant differences in all the sensory texture attributes between the samples. E1 and E3 had the highest elasticity followed by E4 and E2. Looking at firmness E1, E2, and E3 had higher values than E4, which had a higher protein content. This correlated well with the result from the texture analysis, where E4 had the lowest stress value. E2 had the highest fracturability, while E1 and E3 had the lowest fracturability. E2 was described as grainy in structure, which could affect this value. Chewiness was highest for E1 and E3, E2 had a lower chewiness followed by E4. This could correlate with the speed of dissolving where E1 had the lowest value, which could indicate a slow dissolving of pea protein, leading to a chewy structure.

TABLE 2: sensory results and ANOVA pairwise comparison for the sensory descriptive analysis of prototypes of plant-based cheeses E1-E4 (See Table 1 for sample composition). Values are means of the attribute description. The P-value indicated if there were differences between the samples.

Attribute	E1	E2	E3	E4	P-value
Elastic/bouncy	9.3 a*	6.4 b	8.7 a	7.9 ab	0.04
Firmness	9.4 a	7.8 a	8.3 a	5.9 b	<0.0001
Fracturability	5.9 c	12.1 a	6.8 bc	8.6 b	<0.0001
Chewiness	8.4 a	5.4 b	8.0 a	5.3 b	<0.0001
Fast dissolving	7.2 b	9.4 a	7.9 ab	9.4 a	0.04

*different letters in a row indicate a significant difference between the mean scores

Microstructure

The microstructure of the samples also revealed differences, **Figure 2**. Common for all samples was many small fragments of protein and not a connected network structure.

The sample differing most from the remaining microstructure was E2, here bigger protein structures were observed. The protein source in this sample was different from the remaining as it contained the pea-faba mix, which apparently resulted in these bigger and more elongated structures. The larger structures observed in E2 could correlate with the perceived graininess described in the sensory evaluation. This sample was also described as less elastic and chewy and with a high fracturability. This might be due to a matrix disruption by the larger protein fragments.

CONCLUSION

A range of plant-based imitation cheeses was produced to mimic a traditional Danish semi-hard cheese. Combining proteins from pea and chlorella had a positive effect on the textural parameters, whereas a combination of pea protein and a pea-faba bean mix had a negative effect on the measured textural and sensory described parameters. This could be correlated with differences in microstructures of the proteins. Furthermore, at some point, an increased addition of protein gave a softer product.

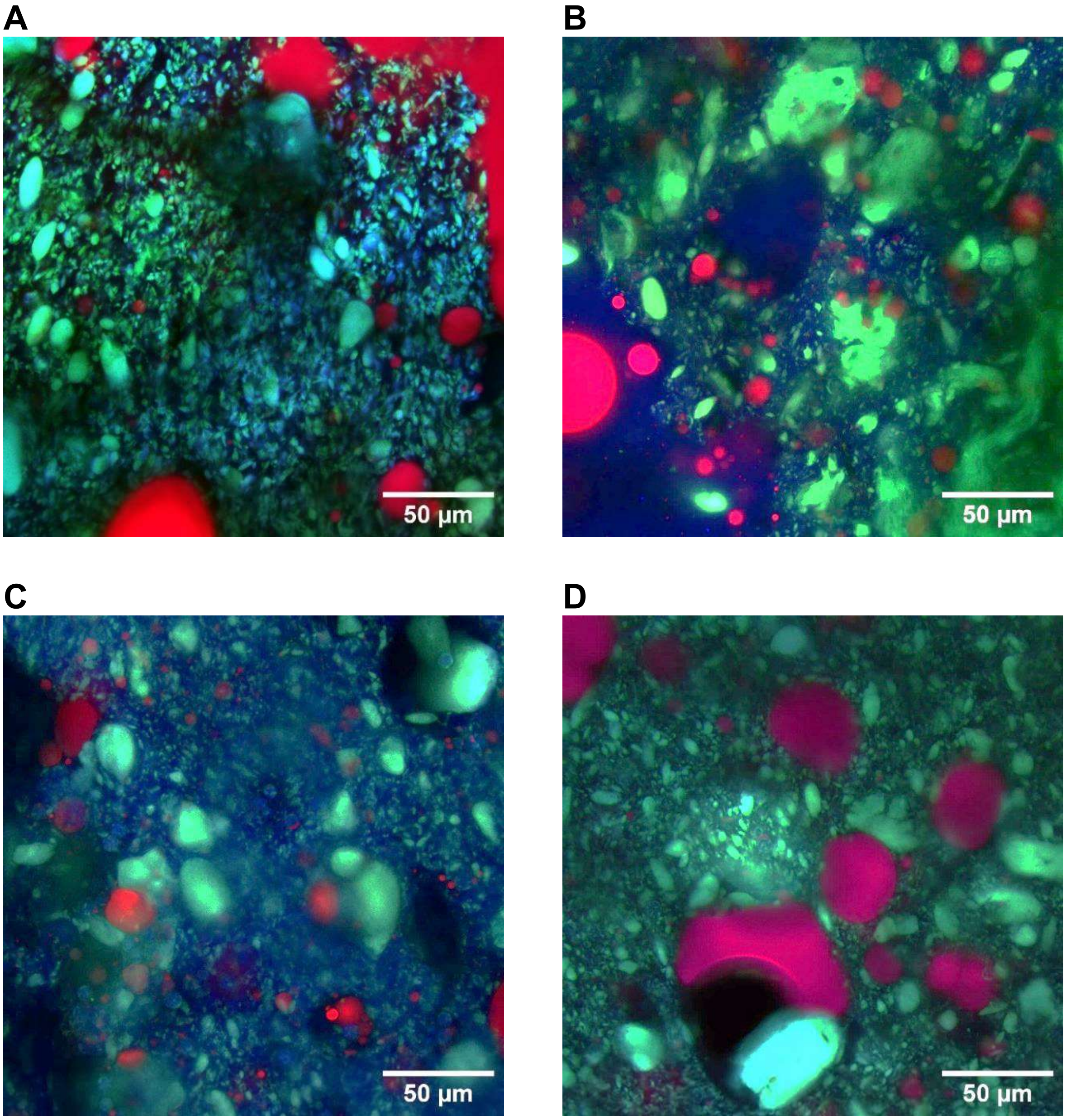

FIGURE 2: Representative Confocal Laser Scanning Microscopy images of E1(A), E2 (B), E3 (C), and E4 (D) (See Table 1 for sample composition). Green: Fast Green labelled protein, blue: FITC labelled starch, red: Nile Red labelled fat.

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