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Development and comparative analysis of protein-polyphenol-fibre bars as nutritional supplements suitable for healthy senior consumers

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Abstract. The number of elderly people is steadily increasing in developing countries though the specific age-related challenges of nutrition fail to get properly addressed in the case of senior citizens. Accordingly, we have developed protein-polyphenol dietary fibre (PPF) bars using two kinds of protein mixtures (1 and 2) and some food additives, such as the banana powder, freeze-dried strawberries, coconut powder, Dutch cacao powder, and vanilla cookies, as they can interfere with the texture of bars and the flavour as well. The used food additives are also a source of polyphenols and dietary fibres that would enhance the nutritive values of the bars. The texture properties, such as hardness and cutting force, were assessed, and the results indicated a significant difference ($P < 0.05$) among the bars, offering important hints about their suitability for the elderly. Also, significant differences were observed for the polyphenol content of the bars that would stress their increased nutritional relevance too. On average, the sensorial evaluation showed the developed bars of moderate acceptability, while Bar 3 and 6 had

the highest scores for colour, texture, flavour, and aroma. Conversely, Bar 1 recorded the lowest values for all assessed criteria.
Interestingly, Bar 3 with freeze-dried strawberries and Bar 6 with Dutch cacao powder were the most appreciated flavours and contained in the range of 25–28% protein, 17–23% carbohydrate, 15–21% lipids, and 15–23% dietary fibre, which also indicates their nutritionally balanced nature. Furthermore, the above-mentioned macronutrient content ensures approximately 400 Kcal/100g per PPF bar, while through their polyphenol and flavonoid yield their health-promoting effect gets substantiated.

Keywords and phrases: protein bars, senior consumer, nutritional supplement, antioxidant activity, texture profile analysis

1. Introduction

Population ageing is becoming an important issue with relevant societal and economic implications. The world population in mid-2022 reached 7,963 million, while the people over the age of 65 were representing 10% of them (*Population Reference Bureau*, Datasheet 2022). At the same time, Europe's population had grown to 742 million, and 19% of the population was over the age of 65.

Moreover, as we grow older, our health condition seems to deteriorate due to inflammageing, a phenomenon featuring increased levels of serum-specific inflammatory markers, which are associated with a high susceptibility to different chronic diseases and premature death (*Ferrucci & Fabbri*, 2018).

In normal circumstances, as adults advance in age, their nutrient needs change, so elderly people need more and/or different nutrients than younger adults, speaking in general terms (*Panda & Booth*, 2022). Additionally, chronic illnesses, medications, and a decrease in physical activity can all increase the risk of nutrient deficiencies or unique requirements *(Baugreet et al.*, 2017). The predicted rise in the elderly cohort represents an opportunity and an obligation to develop optimized, high-protein-containing foodstuffs that could overcome the incidence of age-related conditions such as sarcopenia, one of the most common age-related problems (*Dao*, 2020). Consumption of at least 1.0 to 1.2 g protein/kg/bodyweight/day is recommended for healthy older adults aged 65+ in Europe (*Bauer et al.*, 2013). Moreover, besides proteins, it is equally important to pay attention to the polyphenol intake, as their health-promoting and anti-ageing effects have been demonstrated (*Yessenkyzy et al.*, 2020). Polyphenols can neutralize free radical species and chelate redox-active transition metal ions, all having an implicit antioxidant effect. Furthermore, they can modulate the expression of pro- and anti-apoptotic genes and activate genes implicated in the antioxidant stress response via the Keap1-Nrf2-ARE signalling pathway (*Liu et al.*, 2022). Polyphenols also affect mitochondrial functions, prevent protein aggregation, and induce autophagy having a caloric restriction mimetic effect, and they can be used to prevent or counteract many age-related diseases such as inflammation, diabetes, cancer, and neurodegeneration (*Madeo et al.*, 2019). Polyphenols can also interact with the microbiota–gut–brain axis affecting the ageing brain functionality (*Sarubbo et al.*, 2023).

In recent times, nutritional protein bars have become increasingly popular worldwide due to their convenience and ease of consumption (*Goodbody*, 2013). Typically, they are made from a blend of ingredients like oats, nuts, dried fruits, and protein, providing a nutrient-dense snack or meal option. Protein bars are generally low in calories and fat, making them a healthier choice for snacking in case of children and middle-aged people. Moreover, they are available in various flavours, offering the above-mentioned consumers a wide range of options to choose from (*Padmashree et al.*, 2012). Lately, there has been a rising trend in the utilization of plant proteins as a cost-effective and adaptable substitute for animal-derived sources in human diets. Furthermore, the incorporation of plant proteins into various food uses, including the production of high-protein bars, has the potential to enhance the appeal of these items to individuals following vegan, vegetarian, or active lifestyles (*Sá et al.*, 2020). Overall, rice protein is a good option for people who are looking for a plant-based protein source that is low in carbohydrates and fat. However, it may not provide the same range of nutrients as some other plant-type protein sources, so it is important to consume a diversified diet that includes a variety of protein sources. Moreover, the hydrolysis of proteins with proteases would produce many potential peptide sequences and amino acids providing plenty of nutritive properties. It could also enhance the antioxidative properties of native proteins by attacking the peptide bonds in the interior of polypeptide chains producing a range of polypeptides that differ in molecular weight or amino acid sequences (*Phongthai et al.*, 2017). Further to rice, pea protein has attracted significant attention due to its minimal allergenic potential, exceptional nutritional value, ready availability, and affordability similar to other plant proteins. It contains high levels of lysine but tends to be limited to methionine and tryptophan. Hence, pea proteins are frequently paired with cereal grains because they offer a complementary essential amino acid profile, addressing the typical deficiency in lysine found in cereal proteins, but they contain higher levels of sulphur amino acids (methionine, cysteine) (*Lam et al.*, 2018). Besides rice and pea, the sunflower protein exhibits promising potential as a functional protein owing to its favourable solubility characteristics. Typically derived as a by-product of oil extraction, it often undergoes denaturation during processing, resulting in diminished solubility and functionality. However, when protein fractions are isolated without denaturation, sunflower proteins have the capacity to display solubility across a range of ionic strength and pH levels, making them suitable to provide amino acids (*Ermiş & Karasu*, 2020). Another source for proteins is the hemp whose protein pool features several health-promoting properties, e.g. they influence the angiotensin I-converting enzyme and acetylcholinesterase inhibitions (*Wang & Xiong*, 2019). The hemp protein extract shows antioxidant, hypocholesterolaemia, and hypoglycaemic effects, having significant amounts of arginine and glutamine.

Besides proteins and polyphenols, another important dietary component that has significant relevance for antiaging are the dietary fibres (*Yu et al.*, 2022). The observed and/or recommended intake of dietary fibres varies among people with different cultural backgrounds, so the Iranian elderly would have 25 g/day (*Safarnavadeh et al.*, 2023), Slovenian elderly people consume on average 22.4 g/day (*Seljak et al.*, 2021), while in a USA study people were ingesting 11–21 g/day (*Shivakoti et al.*, 2022). The fibre intake has been correlated with the gastrointestinal and mental health by defining the microbiota–gut–brain axis (*Berding et al.*, 2021), while the mentioned axis implications in neurodegenerative and neuropsychiatric disorders are currently studied intensively (*Bicknell et al.*, 2023; *Shobeiri et al.*, 2022).

It seems, therefore, likely that senior citizens have specific requirements when it comes to healthy nutrition that aids prevention of inflammageing and chronic diseases. It is also important to bear in mind that the maintenance of physical, physiological, and cognitive activities at any time in our lives is an extraordinarily complex task that would require not only the implication of conscious consumers themselves but healthcare professionals too. However, the collaboration of nutritionists, food scientists, and food manufacturers would be more than welcome since they are also responsible for providing suitable foodstuff for people including the elderly (*Alden-Nieminen et al.*, 2009; *Tedre & Pehkonen*, 2014).

The objective of the currently reported study was to develop some protein-, polyphenol-, and dietary-fibre-containing foodstuff that we had named PPF bars and tested them for selected structural, physicochemical, and nutritional parameters. We also evaluated the effects of different protein sources (sunflower, rice, hemp, and pea) in the context of the PPF bar's acceptability by a larger cohort of consumers. We must admit that the presented results represent the initial phase of a larger project, which is meant to result in foodstuff that would support more efficiently the special nutritional requirements of healthy senior people.

2. Materials and methods

All raw materials, such as protein powders, vegetable oil (rapeseed oil, sunflower oil), coconut powder, Dutch cocoa powder, cookies, vanilla powder, maltodextrin, soy lecithin, freeze-dried strawberries and bananas, were purchased from the local markets.

The soy protein isolate, pea protein isolate, rice protein concentrate, sunflower protein, and hemp protein were purchased from an organic shop and were produced by Nutriversum Ltd., Hungary; the glucose syrup (Dextrose Equivalent "DE 38") was a product of KALL Ltd., Hungary, the vegetable oils (rapeseed oil, sunflower oil) were from Bellasan Ltd. (ALDI, Hungary), maltodextrin from VitalTrend Ltd., Hungary, soy lecithin was supplied by Brenntag Ltd., Hungary, natural vanilla aroma in powder by Dr. Oetker Ltd., Hungary cookies by Győri Ltd., Mondelez Hungary, Dutch cacao powder by Dr. Oetker, Hungary, freeze-dried strawberries by Viblance Ltd., Hungary, and coconut powder by Happy Harvest Ltd. (ALDI, Hungary).

The technical and food safety aspects of the products, such as the batch numbers, physicochemical composition, presence of recognized food allergens, sensory characteristics (such as appearance, flavour, and aroma), microbial content, and shelf life, were all checked as well.

Banana powder preparation

At the local market, fully ripe bananas were obtained and sliced into 0.5-cm-thick pieces with the peel intact. To minimize enzymatic browning, the banana slices were immersed in a 10% citric acid solution for 10 minutes. Subsequently, the peel was removed, and the banana slices were air-dried to reduce excess moisture. The dried banana slices were then placed in a cabinet dryer at 60°C for 5 hours, after which they were ground using a Panasonic Mixer Grinder MX-AC555 (India) and sieved through a 30-mesh screen (0.595 mm) (Cole-Parmer, Germany) to obtain a fine flour. Finally, the banana flour was vacuum-sealed for future applications and stored at a 4°C fridge until being used (*Ovando-Martinez M et al.*, 2009).

Preparation of Protein Bars

The optimal amounts of banana powder, coconut flour, Dutch cocoa powder, Oreo cookies, freeze-dried strawberries, salt, and lecithin were determined through trial-and-error methods to achieve the desired colour and texture of the bars. Additionally, the remaining ingredients were selected based on consumer preferences as determined by a survey (data not presented). Once all the ingredients for the recipe were combined, the resulting mixture was placed into silicone moulds and cooled under refrigeration conditions at 4°C for 4 hours. The bars were then coated with chocolate and cooled. Each bar, weighing 40 g, was cut into a rectangular shape and placed in metalized polyester polyethylene (MET-PPE) packaging, which was then sealed. The formulations of the bars were adjusted so that consuming one bar per day could provide the elderly with approximately 23% and 25% of their recommended daily allowance (RDA) of proteins and calories respectively. The rough composition (protein mixture and flavour-conferring ingredient) of PPF bars is presented in *Table 1*. There were two protein mixtures used for the formulation of recipes: Mixture 1, which included pea protein isolate and rice protein, and Mixture 2 containing pea protein isolate, rice protein, hemp protein, and sunflower protein. In *Table 1*, there are also indicated the flavouring ingredients that could also contain important macro- and micronutrients together with phytonutrients and dietary fibres.

Table 1. Rough composition of PPF formula

Bar symbol	Protein mixture and flavour
Bar 1	Mix 1 + Banana powder
Bar 2	Mix 1 + Dutch cacao powder
Bar 3	Mix 1 + Freeze-dried strawberries
Bar 4	Mix 1+ Vanilla and cookies
Bar 5	Mix 2 + Coconut powder
Bar 6	Mix 2 + Dutch cacao powder
Bar 7	Mix 2 + Freeze-dried strawberries
Bar 8	Mix 2 + Vanilla and cookies

Proximate composition analysis and caloric value determination

The composition of PPF bars including moisture, protein, and lipid contents were determined according to the prescribed methods of AOAC (*Association of Analytical Chemists*, 2019), and the results were expressed on a dry-weight basis. The total caloric values (kcal) of the ingredients and samples of final products were calculated as mentioned by *AOAC* (2019), according to the following equation:

$$\text{Energy (kcal)} = [\text{Protein (g)} \times 4] + [\text{Carbohydrates (g)} \times 4] + [\text{Fat (g)} \times 9]. \qquad (1)$$

The determination of the total phenolic and flavonoid content

Determination of the total polyphenol content

Total phenolic content (TPC) was measured by Folin–Ciocâlteu's spectrophotometric method at 760 nm, according to *Lamuela-Raventós* (2018). The content of all phenolic compounds in the tested samples were determined using a calibration curve, for which gallic acid is used (mg GAE /100g). All analyses were performed in triplicate, and the results were statistically evaluated by Excel, a Microsoft Office software program.

Determination of flavonoid content

Total flavonoid content (TFC) was measured spectrophotometrically at 510 nm according to *Seifu et al.* (2017). The content of all flavonoid compounds in the tested samples were determined using the calibration curve, for which catechin was used. The result is given in terms of mg catechin/100 g product. All analyses were performed in triplicate and the results were statistically evaluated by Excel, a Microsoft Office software program.

Texture analysis

A texture analyser, TA (TA – XT2 plus, Stable Micro Systems, Surrey, U.K) was used to measure the hardness of the PPF bar samples as described earlier (*Yuan & Chang*, 2007).

Penetration tests

A single-penetration force-versus-time program was used to compress along the sample thickness at a test speed of 0.50 mm/s and return to its original position. The original clearance between the probe and the base in the machine's load cell was 0.5 mm, so when the probe moved down, it would compress the test sample held horizontally against the base to 0.500 mm, and the hold time was 30 sec. The program software was set to move the probe at 1.0 mm/sec for the pre-test and at 10.00 mm/sec for the post-test phase. A stainless steel probe with a diameter of 10 mm (P/5) was used to compress one sample. The peak force indicated by the force time curve was taken as the maximum compressive force/hardness.

Cutting/shearing tests

The cutting force of the of PPF bars was measured using Texture Analyser (TA-XT2 plus); a single-cutting force-versus-time program was used to compress along the sample thickness at a test speed of 2.00 mm/s and return to its original position. The original clearance between the probe and the base in the machine's load cell was 0.5 mm, so when the probe moved down, it would cut the test sample held horizontally against the base to a distance of 20 mm. The program software was set to move the probe at 1.0 mm/sec for the pre-test and at 10.00 mm/sec for the post-test phase. A stainless steel blade set with knife (HDP/BSK) comprising a Warner Bratzler blade (a reversible blade with knife edge) with a slotted blade insert and a blade holder was used to cut one sample. The cutting curve was obtained by recording the maximum force the blade needs to cut the sample completely. The results were based on the maximum peak (maximum force) resulting from the shear stress.

Sensory evaluation

Sensory evaluation was performed by trained panellists who were experienced in sensory evaluation using the 9-point hedonic scale. The panellists were asked to assess the product based on various criteria, such as appearances, flavour, aroma, colour, texture, and overall acceptability, by choosing and marking one of nine alternatives (ranging from 1 = like extremely to 9 = dislike extremely) according to *Banach et al.* (2014). Instructions were provided before they evaluated the PPF bars. During tasting sessions, samples were presented randomly to panellists. Eight coded samples were presented to the participants. They were instructed to rinse their palate with water between samples. Samples were portioned into equal weights, placed in plastic plates, and kept at room temperature (22 ± 1 °C) for 30 min before analysis. The evaluations were carried out in two sessions.

Statistical analysis

The statistical analysis was performed with the help of one-way analysis of variance (ANOVA) using Minitab statistical analysis software. Differences among obtained means were tested by Tukey's honestly significant difference test (Tukey's HSD). Results are expressed as means ± standard deviation of replicated samples.

3. Results and discussion

The development of PPF bars was carried out as described in the *Materials and methods* section. There have been obtained eight types of bars of which samples 1–4 contained Protein Mix 1 (pea and rice protein isolates in equal amounts) and samples 5–8 having Protein Mix 2 (equal amounts of pea protein isolate, rice protein, hemp protein, and sunflower protein extracts). The detailed composition of the eight PPF bars is shown in *Table 2*. In this table, all the ingredients are indicated, and many of them are expected to confer specific features to every sample. Among these ingredients, the so-called flavours, i.e. the banana powder, coconut powder, Dutch cocoa powder, cookies, freeze-dried strawberries, soy lecithin, and vanilla, were the most relevant. The maltodextrin was not of the resistant type, and its inclusion in the formulation of PPF bars had the purpose of facilitating the formation of an internal texture structure and sensorial quality that appeared more palatable for any age-specific consumer, including the elderly. It was our best intention to pay attention to the age-specific aspects of saliva that are crucial to the oral processing of food and are subsequently also related to sensory and textural experience (*Xu et al.*, 2019).

Table 2. The formulation of the PPF bars

Ingredients (g/100 g)	**Bar 1**	**Bar 2**	**Bar 3**	**Bar 4**	**Bar 5**	**Bar 6**	**Bar 7**	**Bar 8**
Protein Mix 1	33.00	33.00	33.00	33.00	0.0	0.0	0.0	0.0
Protein Mix 2	0.0	0.0	0.0	0.0	33.00	33.00	33.00	33.00
Banana powder	6.23	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Coconut powder	0.0	0.0	0.0	0.0	6.23	0.0	0.0	0.0
Dutch Cocoa powder	0.0	6.23	0.0	0.0	0.0	6.23	0.0	0.0
Cookies	0.0	0.0	0.0	10.23	0.0	0.0	0.0	10.23
Freeze-dried strawberries	0.0	0.0	10.23	0.0	0.0	0.0	10.23	0.0
Sunflower oil	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Glucose syrup	31.82	31.82	27.82	27.82	31.82	31.82	27.82	27.82
Rapeseed oil	13.64	13.64	13.64	13.64	13.64	13.64	13.64	13.64
Maltodextrin	5.45	5.45	5.45	5.45	5.45	5.45	5.45	5.45
Water	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Soy lecithin	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85

Ingredients (g/100 g)	Bar 1	Bar 2	Bar 3	Bar 4	Bar 5	Bar 6	Bar 7	Bar 8
Vanilla	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Salt	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

However, in the future, the use of resistant maltodextrin should also be considered, which is a soluble dietary fibre ingredient whose physiological functions are well studied, and its implications in regulating blood glucose levels and serum lipids are demonstrated (*Astina & Sapwarobol*, 2019).

The nutritional facts: Per/100 g bar

Next, we have analysed the proximate composition of the developed PPF bars, which are presented in *Table 3*. The assessment of the nutritional composition comprised parameters such as fat, carbohydrates, fibres, protein, and total caloric content, and the obtained data refers to 100 g of a given PPF bar.

Table 3. The proximate composition data of developed PPF bars

Bar symbol	Fat (g/100g)	Carbohydrate (g/100g)	Fibre (g/100g)	Protein (g/100g)	Energy, Kcal
	Mean + St. Dev.	Mean + St. Dev.	Mean + St. Dev.	Mean + St. Dev.	Mean + St. Dev.
Bar 1	15.27 ± 0.83[b]	23.52 ± 0.55 [a]	15.97 ± 1.01[b]	28.02 ± 0.03 [a]	341.38 ± 4.12[a]
Bar 2	20.73 ± 2.04 [a]	17.55 ± 0.623 [b c]	23.67 ± 0.91[a]	26.83 ± 0.96[a]	354.37 ± 2.01[a]
Bar 3	20.50 ± 0.79 [ab]	19.27 ± 0.95 [b]	23.53 ± 0.56[a]	25.1 ± 2.36[a]	321.08 ± 1.00[a]
Bar 4	19.37 ± 0.77 [ab]	19.33 ± 0.62 [b]	23.33 ± 1.78 [a]	26.75 ± 0.86[a]	341.35 ± 3.15[a]
Bar 5	20.54 ± 1.81[a b]	15.54 ± 0.78 c	21.84 ± 2.62 [a]	27.06 ± 0.97[a]	387.86 ± 3.57[a]
Bar 6	21.0 ± 1.83 [a]	22.69 ± 0.34[a]	21.98 ± 1.66[a]	28.63 ± 0.77[a]	368.06 ± 4.00 [a]
Bar 7	20.40 ± 1.71 [ab]	18.38 ± 1.08[b]	23.12 ± 0.73 [a]	27.32 ± 0.29[a]	377.80 ± 0.17[a]
Bar 8	21.43 ± 3.71 [a]	18.36 ± 0.53 [b]	23.01 ± 0.40[a]	27.46 ± 0.73[a]	376.85 ± 2.41[a]

Notes: * Values are expressed as mean ± SD. For each column, values that contain different letters in superscripts were significantly different (P ≤ 0.05).

The fat content ranged from 15.2 g to 21.4 g. The results showed that the highest ($p < 0.05$) fat content (21.43 ± 3.71 g) was recorded for the sample *Bar 8* followed by the samples *Bar 6* and *Bar 2* (21.0 ± 1.83 g, 20.73 ± 2.04 g), and this fact could be due to the Dutch cocoa powder content of the mentioned bars.

The values of the available carbohydrate content in the samples were found in the range of 15.54–23.52 g and varied significantly ($p < 0.05$) among products. The highest carbohydrate content (23.52 ± 0.552 g) was recorded in the case of *Bar 1*, which featured the lowest fibre content (15.97 ± 1.01 g) too.

The fibre content varied between 15.53 and 23.52 g, which is a relatively large interval that could meet to some extent the optimal fibre intake of adult people, which is largely dependent on cultural and economic considerations. However, in order to increase the suitability of PPF bars for elderly people, we would increase the dietary fibre content of such bars, and attempts will be made to accommodate the psyllium husk and the resistant maltodextrin. The resistant maltodextrin (*Astina & Sapwarobol*, 2019) and psyllium husk (*Bacha et al.*, 2022; *Chen et* al., 2022) have known beneficial implications for adults' health conditions.

The PPF-bar-related protein content and total caloric value seemed to look relatively constant, as there were no significant differences detected between the samples. Despite the roughly 27 g of protein that is delivered by a PPF bar, the amino acid and especially the essential versus non-essential amino acid content and their bioavailability or bioaccessibility remain an open question. Moreover, it would be important to consider the use of whey protein isolate to further improve the bioavailability of protein sources in the case of future PPF bars since the amino acid blood concentration following whey protein ingestion was higher than pea and cricket proteins (*Lanng et al.,* 2023).

Total phenolic and flavonoid content

The phenolic and flavonoid content were determined as described in the *Materials and methods* section. The TPC and TFC data were significantly different ($p < 0.05$) among the analysed PPF bars (see *Table 4*).

Table 4. The total polyphenol and flavonoid content of the PPF bars

Bar symbol	**Polyphenols (mg GAE/100g)**	**Flavonoids (mg CE/100g)**
	Mean + St. Dev.	*Mean + St. Dev.*
Bar 1	86.39 ± 0.60 [b]	85.43 ± 5.11 [a]
Bar 2	103.04 ± 3.58 [a]	78.85 ± 3.80 [a b c]
Bar 3	82.36 ± 1.63 [b]	75.58 ± 1.20 [b c]
Bar 4	90.14 ± 5.80 [b]	82.28 ± 0.39 [a b]
Bar 5	66.89 ± 1.00 [c]	67.46 ± 3.79 [c d]
Bar 6	81.93 ± 1.79 [b]	54.35 ± 0.59 [d]
Bar 7	88.02 ± 2.56 [b]	87.09 ± 5.36 [a]
Bar 8	73.02 ± 0.97 [c]	57.00 ± 3.67 [d]

Notes: * Values are expressed as mean ± SD. For each column, values that contain different letters in superscripts were significantly different ($P \leq 0.05$).

Interestingly, among the PPF bars, the highest TPC values were featured by *Bar 2* (103.04 ± 3.58 mg GAE/100g) and *Bar 4* (90.14 ± 5.80 mg GAE/100g), while the lowest polyphenol content was found in *Bar 5* (66.89 ± 1mg GAE/100g) and *Bar 8* (73.02 ± 0.97 mg GAE/100g). The higher content of TPC in PPF, such as in *Bar 2*, where cacao powder was added, is reasonable since the cacao-specific increased phenolic content is known (*Del Rio et al.*, 2011; *Urbańska* & *Kowalska*, 2019). However, the increased TPC in *Bar 4* looks a bit puzzling, but most likely it is due to the differences that might exist among the used protein mixtures.

The highest content of TFC was found in sample *Bar 7* (87.09 ± 5.36 mg CE/100g) followed by *Bar 1* (85.43 ± 5.11 mg CE/100g). The lowest content of TFC was found in sample *Bar 6* (54.346 ± 0.595 mg CE/100g) and *Bar 8* (57.00 ± 3.67 mg CE/100g). The higher content of TFC in sample *Bar 7* is somehow puzzling because if it was due to the strawberry, then we should have seen an increased TFC also in case of *Bar 3*. Nevertheless, the excelling TFC value in *Bar 1* could be potentially attributed to the presence of the banana, whose phytonutrient and flavonoid content was extensively studied (*Mondal et al.*, 2021). Another interesting feature of the TFC is somehow related to the Dutch cacao powder containing *Bar 2* and *Bar 6*, where the differences in TFC values could be put in the context of the different protein mixtures seen in these two PPF bars. These observations regarding the TPC and TFC of the PPF bars and other peculiarities would suggest the relevance of protein mixtures that might confer some additional properties related to the efficiency of polyphenol and flavonoid extractions and bioavailability.

The texture analysis

The analysis of the structure and texture are of great importance because it can offer important information about the chewability of the PPF bars that could affect not just the breakdown pattern but also the digestibility and ultimately bioavailability of nutrients. Undoubtedly, the mastication of any foodstuff and swallowing mechanisms have been shown to adapt and change during the lifetime of humans (*Cichero*, 2017). It has also been demonstrated that the hardness of food affects mastication, and the mandibular movement increases gradually as the food gets harder (*Komino & Shiga*, 2017). Similarly, physiological deterioration like aging, poor dental status, and reduced tongue pressure would result in an affected masticatory performance (MP) that makes chewing more difficult (*Park et al.*, 2022). The MP is also referred to as chewing performance and is the ability of an individual's masticatory system to reduce food to small particles to be swallowed. Experiments have demonstrated that the texture of food would affect the MP, so the number of chews and chewing time increases with the hardness of food though in the case of older people, the MP appears significantly influenced by the nature of dentures (complete versus natural), and the older people with natural teeth exhibit higher tongue pressure than those with complete dentures. All these observations suggest that the analyses of food texture by assessing the average hardness and maximum cutting forces can offer valuable data concerning the suitability of the developed PPF bars with respect to a specific consumer niche. The analysis of the average hardness and maximum cutting forces were performed as described in the *Materials and methods* section. The obtained results are shown in *Table 5*.

Table 5. The hardness and cutting forces of the PPF bars

Sample	Used protein mix	Hardness force (N)	Cutting resistance force (N)
		Mean + St. Dev.	Mean + St. Dev.
Bar 1	Mix1	43.64 ± 6.55 [a]	34.39 ± 0.74 [a]
Bar 2	Mix1	7.87 ± 0.78 [b c]	11.43 ± 0.48 [c d]
Bar 3	Mix1	10.47 ± 2.09 [b c]	16.24 ± 0.50 [b]
Bar 4	Mix1	8.094 ± 0.08 [b c]	11.34 ± 0.33 [d]
Bar 5	Mix2	12.51 ± 0.98 [b]	13.47 ± 0.53 [c]
Bar 6	Mix2	6.43 ± 0.21 [b c]	16.16 ± 1.53 [b]
Bar 7	Mix2	7.84 ± 1.21 [b c]	10.06 ± 0.53 [d]
Bar 8	Mix2	4.13 ± 0.66 [c]	11.34 ± 0.33 [e]

Notes: * Values are expressed as mean ± SD. For each column, values that contain different letters in superscripts were significantly different ($P \leq 0.05$).

Typically, the hardness of high-protein bars is notably increased and tends to rise with the introduction of additional protein (*Li et al.*, 2008). The highest value of the hardness was found for *Bar 1* (43.64 ± 6.55 N) followed by *Bar 5* (12.51 ± 0.98 N), while the lowest hardness value was seen for *Bar 8* (4.13 ± 0.66 N). Noticeably, other samples, such as *Bar 6*, *Bar 7*, and *Bar 2*, were scoring lower values but nearer to *Bar 8*, while *Bar 3* was much closer to the increased value of *Bar 5*.

The observed variances in the hardness of the PPF bars could be related to the differences in the constitutive ingredients. The elevated hardness of sample *Bar 1* could be only partly due to the use of protein mixture 1 (pea protein and rice protein) because this protein mix being used in the formulations of *Bar 2*, *Bar 3*, and *Bar 4* does not result in exceedingly increased hardness values. It is also possible that the banana powder used for the formulation of *Bar 1* confers an elevated rigidity to the texture of this PPF bar. Conversely, the lowest hardness force observed for sample *Bar 8* might be due to protein mixture 2 (pea, rice, hemp, and sunflower), which can result in a more diverse and flexible protein network with a more tender texture (*Zahari et al.*, 2021).

With respect to the protein implication in food texture formation, it has been shown that the different amino acid profiles could influence the formation of the protein networks (*Małecki et al.*, 2020). Conversely, the lowest hardness force observed for sample *Bar 8* might be due to protein mixture 2 (pea, rice, hemp, and sunflower), which can result in a more diverse and flexible protein network with a more tender texture. For example, lysine and arginine are positively charged at neutral pH, which means they can form strong hydrogen bonds with other amino acids that are negatively charged. These hydrogen bonds can contribute to the formation of a rigid protein network and a harder texture *(Lu et al.*, 2019). Cysteine and methionine are amino acids that are relatively abundant in rice protein extract and can form disulphide bonds with other cysteine residues. Disulphide bonds are strong and can stabilize the protein network. However, too many disulphide bonds can also make the protein bars adopt a hard texture (*Lu et al.*, 2019). Additionally, the functional properties of the proteins can also affect their ability for network formation. For example, pea protein isolate is known to have a good gel-forming property, which stabilizes the protein network and confers a harder texture. Rice protein isolate, on the other hand, is also known to have good emulsifying property, which can make further ingredients bind together but may not contribute as much to the hardness of a given protein bar (*Phongthai et al.*, 2017).

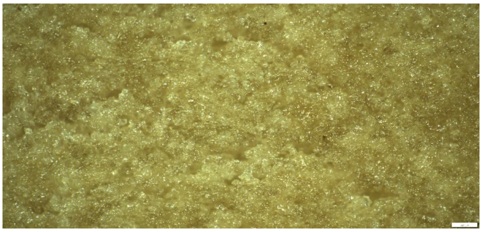
Regarding the maximum cutting force analysis, the results would indicate that *Bar 1* showed an exceedingly high cutting resistance force (34.39 ± 0.74 N), followed by the mostly halved values of *Bar 6* (16.16 ± 1.53 N) and *Bar 3* (16.24 ± 0.50 N) (see *Table 5*).

On the other hand, we found that *Bar 7* had the lowest cutting resistance (11.34 ± 0.33 N) and was closely followed by *Bar 8* (11.34 ± 0.33 N) and *Bar 2* (11.43 ± 0.48 N).

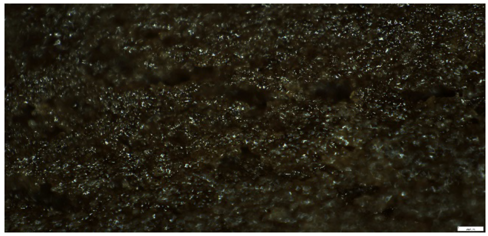
The reduced cutting force observed for *Bar 7*, *Bar 8*, and *Bar 5* might be correlated with the presence of protein mixture 2 in the PPF bars. It seems possible that the use of soluble corn fibre, vegetable glycerin, and chicory root fibre may contribute to a softer texture by increasing the moisture content and reducing the density of the protein network. The above-mentioned ingredients are known to have humectant properties, which means that they can absorb and retain moisture, leading to a softer and tenderer texture (*Małecki et al.*, 2022). Secondly, this is because sunflower oil and lecithin are known to have lubricating and emulsifying properties, which can affect the protein network's ability to form a cohesive structure (*Wang et al.*, 2018).

Furthermore, some of the used flavours may also affect the texture of the PPF bars – for instance, a flavour with a high moisture content, such as fruit, may add moisture to the bar and make it softer. Conversely, a flavour with a dry texture, such as coconut powder, may make the bar harder and drier (*Mazumder et al.*, 2021). In addition, some flavours may contain ingredients that can further modify the texture. For example, a flavour that contains chunks of nuts or fruit may create a chewy texture in the bar. On the other hand, a flavour that contains a liquid or oil-based ingredient may make the bar softer or moister *(Mazumder et al.*, 2021).

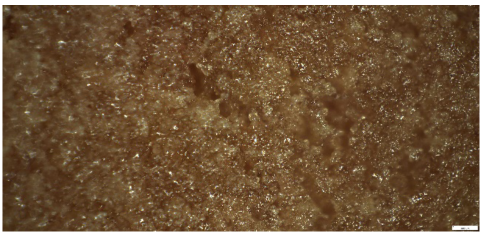
Next to the hardness and cutting resistance analysis, we further assessed the texture outlook by monitoring the microscopic cross-section images of the PPF bars (see *Fig. 1*). Food texture is an important feature of our sense and is meant to describe the foodstuff-generated feeling in the month.



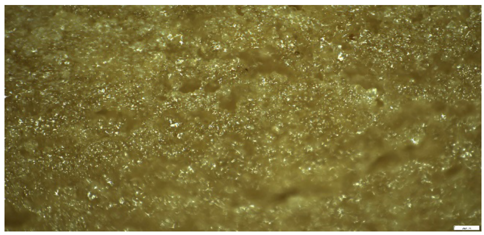
Bar 1



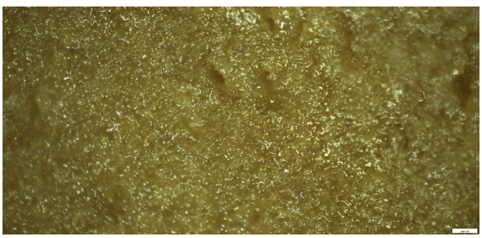
Bar 2



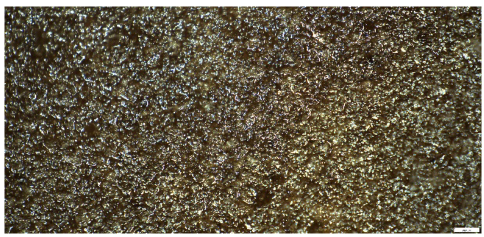
Bar 3



Bar 4

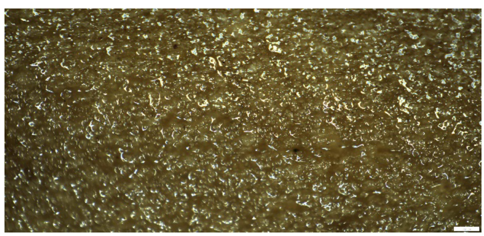


Bar 5



Bar 6

Bar 7



Bar 8

Notes: * The images were taken by Microscope model SZX2-ILLK (Japan); the scale bars represent 200 µm.

Figure 1. Microscopic cross-section of the PPF bars

The micrographic image of the PPF bars denotes a fairly uniform chewy type of texture with an even distribution of the particles, and no crunchy outlook can be inferred. It should be noticed that the texture of *Bar 1* appears with more unevenly distributed particles than all the other bars.

Taken together, the obtained hardness- and cutting-resistance-related results correlate with each other (see *Fig. 2*) and also corroborate the micrographic data of the PPF bars.

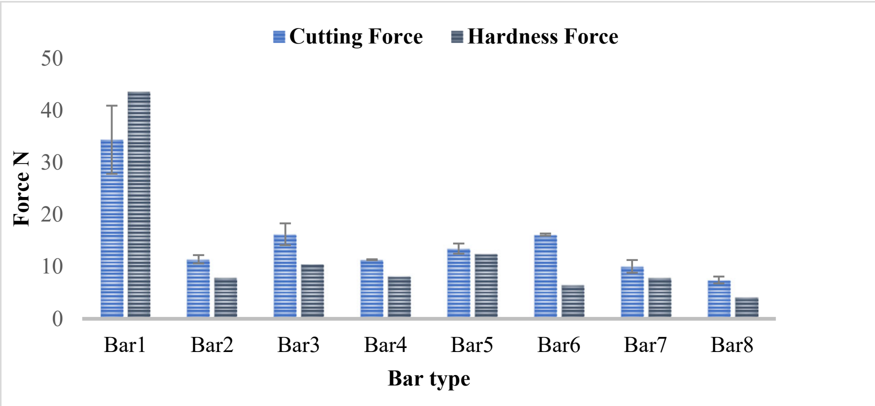


Figure 2. Comparative analysis of cutting and hardness forces data of PPF bars

It is also very important to pinpoint that with the exception of *Bar 1,* all the other, *PPF2–8,* bars are expected not to interfere unfavourably with the mastication performance, and therefore they should score appreciative sensorial grades.

The sensorial evaluation

In order to gain some feedback from customers, we carried out a sensorial evaluation, assessing the appearance, colour, flavour, aroma, texture, and overall acceptability of the PPF bars. The applied method is described in the *Materials and methods* section, while the obtained data are presented in *Fig. 3*. The customer attitudes towards the PPF bars were mostly critical, and they showed great interest in the assessment.

All developed bars were considered acceptable to the participants in terms of appearance. It could be observed that *Bar 6* had the highest scores in terms of appearance, flavour, and overall acceptability, and it noticeably contained protein mixture 2 and the Dutch cacao powder. Also, *Bar 3* was the most preferred by the panellists in terms of flavour, aroma, colour, and texture with mean scores above 7 for most factors. *Bar 3* contains protein mixture 1 and freeze-dried strawberries.

Moreover, *Bar 3* and *Bar 6* scored reduced hardness and cutting resistance force values and seemed to be well-liked by all participants. On the other hand, *Bar 1* had significantly the lowest scores in colour, flavour, aroma, texture, and overall acceptability, which would suggest that this PPF bar has been less palatable or enjoyable to the participants. The low scores of *Bar 1* could be related to its stickiness, less smoothness, and harder consistency, which are features that could be associated with the elevated hardness and cutting resistance values.

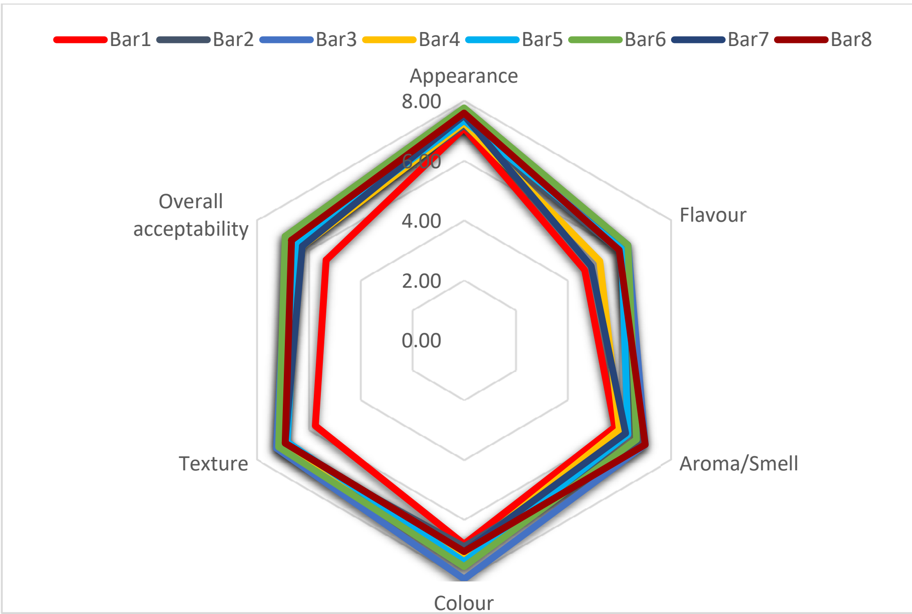


Figure 3. Radar chart of the sensory evaluation of the PPF bars

The sensorial evaluation clearly showed that the overall appearance and texture structure affected the attractiveness and acceptability of the PPF bars. All these observations suggest that some PPF bars, such as *Bar 6* and *Bar 3* containing different protein mixtures and flavours, are suitable for future improvements.

4. Conclusions

Data of the conducted research suggest the influence of the type of protein and food additives used on the textural parameters, nutritional values, and physicochemical parameters. Results showed that the highest polyphenol content values were featured by bars where cacao powder was used, while the highest flavonoid content was found in the bars where cacao powder and banana powder were added. On the other hand, the parameters of texture profile analysis (TPA) and the cutting forces of the developed bars showed significant differences and could be related to the differences in the constitutive ingredients; the data revealed that adding the banana powder to the formula of the bars would lead to a higher value regarding hardness and cutting force. The sensorial evaluation also revealed that adding the freeze-dried strawberries and Dutch cacao powder did enhance the flavour of the bars. Taken together, the developed PPF bars represent a successful beginning in our quest to increase their suitability and health-promoting effects for senior citizens by paying attention to nutrients such as proteins, polyphenols, and dietary fibres.

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