OCL 2023, 30, 24
© J. Presa-Lombardi *et al.*, Published by EDP Sciences, 2023
https://doi.org/10.1051/ocl/2023025

Available online at:
www.ocl-journal.org

RESEARCH ARTICLE OPEN ACCESS

Hemp seed's (*Cannabis Sativa L*) nutritional potential for the development of snack functional foods☆

Joaquín Presa-Lombardi, Florencia García, Maria Belen Gutierrez-Barrutia and Sonia Cozzano*

Universidad Católica del Uruguay, Department of Engineering, Montevideo, Uruguay

Received 30 May 2023 – Accepted 13 October 2023

Abstract – Current trends in the snack bar market emphasize the incorporation of alternative protein sources while simultaneously addressing the criteria of being both "high in fiber" and "high in protein". Hemp seed, the seed of non-psychoactive *Cannabis Sativa L*, stands out as a significant source of protein, dietary fiber, minerals, and unsaturated fats. This study aims to explore the nutritional potential of hemp seed to develop a functional food that responds to the needs of such a market highly demanding of plant-based alternatives. Along with seeds protein nutritional quality analysis and lipid profile characterization, three snack bar samples containing hemp seed as a functional ingredient were formulated, according to nutritional claims regulations. Two products with 20% hemp met sensory acceptability criteria. This study suggests hemp seed as a potential functional food ingredient to meet the demand for plant-based alternatives, offering quality protein, digestive benefits due to its high fiber content and an optimal omega 6 to omega 3 ratio. However, there appears to be an upper limit for hemp seed in product formulations due to sensory issues. This study showed that adding more than 40% to a snack bar significantly reduced consumer acceptability and purchase intent.

Keywords: Hemp seed / functional foods / hemp protein / healthy snacks / alternative protein

Résumé – Potentiel nutritionnel des graines de chanvre (Cannabis Sativa L) pour le développement d'aliments fonctionnels de type snack. Les tendances actuelles sur le marché des barres-collations mettent l'accent sur l'incorporation de sources alternatives de protéines tout en répondant simultanément aux critères d'être à la fois « riches en fibres » et « riches en protéines ». Les graines de chanvre, la graine du *Cannabis Sativa L* non psychoactif, se distinguent comme une source importante de protéines, de fibres alimentaires, de minéraux et de graisses insaturées. Cette étude vise à explorer le potentiel nutritionnel des graines de chanvre pour développer un aliment fonctionnel répondant aux besoins d'un marché très exigeant en alternatives végétales. Parallèlement à l'analyse de la qualité nutritionnelle des protéines des graines et à la caractérisation du profil lipidique, trois échantillons de barres de collation contenant des graines de chanvre comme ingrédient fonctionnel ont été formulés, conformément à la réglementation sur les allégations nutritionnelles. Deux produits contenant 20% de chanvre répondaient aux critères d'acceptabilité sensorielle. Cette étude suggère que les graines de chanvre sont un ingrédient fonctionnel alimentaire potentiel pour répondre à la demande d'alternatives à base de plantes, offrant des protéines de qualité, des bienfaits digestifs grâce à leur teneur élevée en fibres et un rapport optimal entre oméga 6 et oméga 3. Cependant, il semble y avoir une limite supérieure pour les graines de chanvre dans les formulations de produits en raison de problèmes sensoriels. Ainsi, cette étude a montré qu'un ajout de plus de 40 % à une collation en barre réduisait considérablement l'acceptabilité et l'intention d'achat du consommateur.

Mots-clés : Graine de chanvre / aliments fonctionnels / proteine de chanvre / collations saines / proteine alternative

☆ Contribution to the Topical Issue "How oil- and protein- crops can help fight against climate change? / Contribution des oléoprotéagineux à la lutte contre le changement climatique"

*Corresponding author: scozzano@ucu.edu.uy

1 Introduction

Hemp (*Cannabis Sativa L*) belongs to the non-psychoactive industrial variety of the Cannabaceae plant family that contains less than 1% tetrahydrocannabinol or THC (Lachenmeier *et al*., 2004). Some of the main uses of hemp are in the textile industry, both industrial and consumer, and in the production of construction materials or animal feed. However, given the nutritional characteristics of the seed, it can be included as an ingredient for the food industry that can contribute to the rapidly growing market of plant-based alternatives.

Hemp seed is an important source of protein, dietary fiber, vitamins, minerals and unsaturated fats. According to Siano *et al*. (2018) hemp seed contains approximately 25% protein, 38% carbohydrates (35% fiber) and 25% fats. Of the total fats, 70–80% are polyunsaturated and 10% monounsaturated fatty acids, being an exceptional source of essential omega-3 fatty acids. Additionally, its oil has one of the lowest ($\omega6/\omega3$) ratios found in nature (Callaway, 2004), which is considered optimal for human health (Simopoulos, 2002). In addition, hemp seeds have a beneficial content of phenolic compounds, phytosterols and tocopherols (Trovato *et al.,* 2023), as well as high antioxidant capacity (Irakli *et al*., 2019) which are key qualities for functional ingredients.

Regarding one of the primary constituents of hemp seeds, dietary fiber, it is made up of a ratio of 80:20 between insoluble and soluble fiber (Callaway, 2004). The majority (70%) is found in the hull, in fact, when it comes to the nutritional composition of the hulled seed, also known as “hemp heart” the total percentage of fiber drops from 35% to 5–10% (Leonard *et al.,* 2020). The health benefits that come from dietary fiber have already been studied and widely demonstrated. Among the most prominent are the promotion of digestive health, reduced risk of colorectal cancer and cardiovascular disease, largely due to the production of short-chain fatty acids (Anderson *et al.,* 2009; Lattimer *et al.,* 2010).

Due to the growing interest in hemp seed together with plant-based protein alternatives, many studies have analyzed hemp protein. Hemp protein contains all nine essential amino acids, generally with high levels of glutamine and arginine (Leonard *et al.,* 2020). The amino acid profile is comparable to that of soy, but with better enzyme digestibility (Wang *et al.,* 2008). Given its rich amino acid profile, hemp seed is proposed as a good matrix for the development of functional food products with the nutritional claims “source of/high in” protein.

Previous studies have evaluated the use of hemp seeds in pasta (Teterycz *et al*., 2021), bread (Mikulec *et al*., 2019) and it is important to investigate its incorporation in snack bars, being an easily consumed product that is ready-to-eat. Developing functional snack products with acceptable sensory properties represents a food industry priority, in order to improve well-being, nutritional and health status of consumers, while preventing non-communicable diseases (Coello *et al.,* 2022). Additionally, current and emerging trends in the snack bar market suggest two key implications for new product development. First, alternative protein sources, as opposed to more traditional dairy protein, are becoming increasingly popular (Boukid *et al.,* 2022). Second, there is a demand for snack bars that meet both "high in fiber" and "high in protein" nutritional claims. Incorporating hemp seeds, with their favorable nutritional composition, can address both of these product development implications (Boukid *et al.,* 2022).

The aim of this work is to investigate the nutritional potential of non-psychoactive *Cannabis Sativa L.* to develop a functional food that responds to the needs of a market highly demanding of plant-based alternatives.

2 Research materials and methods

2.1 Nutritional value of hemp seed

2.1.1 Nutritional characterization

The proximate composition of Futura 75 hemp seed was determined. Whole hemp seeds were kindly provided by Goland Group (Montevideo, Uruguay). Protein and total dietary fiber (TDF) content were determined according to AOAC methods 984.13 (AOAC International, 2012a) and 985.29 (AOAC International, 2012b), respectively. Fat content was analyzed according to ISO 6492 (International Organization for Standardization, 1999). Moisture content was determined by gravimetric analysis in an oven at 105 °C until constant weight. Ash was determined in a muffle furnace according to ISO 5984 (International Organization for Standardization, 2002, cor.1 2005). Carbohydrate content was calculated by difference.

2.1.2 Amino acid profile and protein nutritional quality

Free amino acid profile was performed by Eurofins Scientific (Food Chemistry Testing) according to Schuster (1988); Barkholt & Jensen (1989). The samples were hydrolyzed in 6 N Hydrochloric acid for 24 hours at approximately 110°C. Phenol was then added to the 6N Hydrochloric acid to prevent halogenation of tyrosine. Cystine and cysteine were converted to S-2-carboxyethylthiocysteine by the addition of dithiodipropionic acid. The samples were analyzed by HPLC after pre-injection derivatization. The primary amino acids were then derivatized with o-phthalaldehyde (OPA) and the secondary amino acids were derivatized with fluorenylmethyl chloroformate (FMOC) before injection. Tryptophan was not detected because the acidic conditions necessary for quantification cause its hydrolysis.

HPLC equipment (Agilent 1200) was equipped with a binary pump (G1312B) and a diode array detector (G1315C). Solutions were injected in a volume of 20 µL in a ZORBAX Eclipse XDB-C18 column (150 mm × 2,1 mm × 3,5 µm) at 40°C. Mobile phase A was composed of 10 mM Na2HPO4 and 10 mM Na2B4O7, pH 8.2, while mobile phase B was acetonitrile: methanol: water (45:45:10, v: v: v). The elution gradient used was (time, %B): 0-0,5 min, 2%; 20 min, 57%; 20,1 min, 100%; 23,5 min, 100%; 23,6 min, 2%; 25 min, end (Flow 0,42 mL/min).

To determine protein quality, the following calculations were conducted:

The Protein Efficiency Ratio (PER) was based on the following three equations (Mir *et al.,* 2019):

$PER_1 = -0{,}684 + (0{,}456 \times Leu) - (0{,}047 \times Pro)$,

$PER_2 = -0{,}468 + (0{,}454 \times Leu) - (0{,}105 \times Tyr)$,

Table 1. Snack bars ingredient proportions, nutrition facts and reached nutritional claims according to each regulation.

Ingredients	S1	S2	S3
Almonds (%)	18,52	22,22	29,41
Dates (%)	12,96	18,52	19,61
Hemp flour (%)	37,04	18,52	19,61
Egg albumin (%)	12,96	22,22	11,76
Chocolate (%)	18,52	18,52	19,61
Nutrition Facts			
Protein (g per 50g)	12,2	13,9	10,6
Fats (g per 50g)	13	8,1	10,1
Carbohydrates (g per 50g)	15,2	10,5	11,5
Fiber (g per 50g)	5,9	3,9	4,3
Energetic Value (kcal per 50g)	202	155	162
Nutritional Claims			
EU	High Fiber High Protein High in omega 3 fatty acids	High Fiber High Protein Source of omega 3 fatty acids	High Fiber High Protein Source of omega 3 fatty acids
Mercosur	High Fiber High Protein Source of omega 3 fatty acids	Source of fiber High protein Source of omega 3 fatty acids	Source of fiber Source of protein Source of omega 3 fatty acids

S1 stands for Sample 1, S2 for Sample 2 and S3 for Sample 3.

$PER_3 = -1{,}816 + (0{,}435 \times Met) + (0{,}78 \times Leu)$,
$+ (0{,}211 \times His) - (0{,}044 \times Tyr)$,

Satiety indicators (SAT) (Greco *et al.*, 2017; Volk *et al.*, 2020):

SAT_1(g/100g of protein)=Leu + Ile + Lys + Thr + Tyr,
SAT_2 (g/100g of protein) = Thr + Tyr.

The essential amino acid index (EAAI) was calculated as the geometrical mean of the ratio of all the EAA in the seed to their content in a highly nutritive reference protein such as whole egg (16).

$$\%EAAI = 100 \times \sqrt[n]{\frac{His_a\left(\frac{g}{100g\ of\ protein}\right) \times \ldots \times Val_a\left(\frac{g}{100g\ of\ protein}\right)}{His_b\left(\frac{g}{100g\ of\ protein}\right) \times \ldots \times Val_b\left(\frac{g}{100g\ of\ protein}\right)}}.$$

Biological Value (BV) was calculated as (Oser, 1959):
BV = EAAI x 1,09–11,7

2.1.3 Fatty acid profile

Identification and quantification of fatty acids was done according to IUPAC 2.301–2.304 (Paquot *et al.*, 2013), using a gas chromatography (equipped with a flame ionization detector (FID) at 260 ∘C, an automatic injector and a 60 m × 0.325 mm × 0.25 µm column. Two µL of samples or standards were injected splitless at 250 ∘C. The carrier gas used was helium at a constant flow rate (1.5 mL/min). The initial column temperature was 50 ∘C and held 2 min, increased to 150 ∘C at the rate of 15 ∘C/min, to 200 ∘C at 5 ∘C/min and increased to 240 ∘C at the rate of 15 ∘C/min and kept for 20 min. The analyses were performed in triplicate.

2.2 Development of the high-nutrient hemp seed bar

Hemp flour

To obtain hemp flour, whole seeds were ground in a KitchenAid coffee grinder to a particle size of 1mm.

Tested food

The following ingredients were used to prepare the snack bars: almonds, egg albumin, dates (purchased on a local market), hemp flour, and chocolate (Dark Belcolade; cocoa mass, sugar, cocoa butter, flavoring: vanilla). Egg albumin was added with the aim to obtain a final product with high protein quality and a balanced amino acid content, as egg white is widely considered a high-quality protein source, which also complements hemp seed's lack of methionine, leucine and isoleucine. Three different formulations were done. Proportion of ingredients is shown in Table 1. To prepare the bars, the first four ingredients were mixed in such a way that a homogeneous paste was obtained, 50-gram bars were formed and refrigerated for 2 hours.

Reaching different nutritional claims (as shown in Table 1) was the criteria for sample formulation. Sample 1 had the highest hemp seed content in order be the only sample to reach both "high in fiber" and "high in protein" local regulation claims, which were better known to consumers conducting the test. The other two samples had the same hemp percentage but differed in protein so as Sample 3 became the only sample which was just "source of..." in both fiber and protein. Nutritional claims were determined by the local Mercosur Technical Regulation ("Tratado de Asunción, el Protocolo de Ouro Preto y las Resoluciones N° 38/98, 56/02, 46/03, 47/03, 31/06 y 48/06

- ☐ Tasty
- ☐ Dry
- ☐ Easy to chew
- ☐ Soft
- ☐ Off - flavor
- ☐ Crumbly
- ☐ Nutritious
- ☐ Fibrous
- ☐ Healthy
- ☐ Hard to chew
- ☐ Mealy
- ☐ Moist
- ☐ Hard

Fig. 1. Check-all-that-apply (CATA) questions for consumers at the sensory analysis.

del Grupo Mercado Común") and the European Commission (Regulation (EU) No 1047/2012).

Table 1 also shows the nutritional composition of each sample S1, S2 and S3, calculated theoretically considering the nutritional composition and proportion (%) of each ingredient.

2.3 Sensory analysis

A sensory evaluation with consumers was conducted for the three different snack bars at Universidad Católica del Uruguay (Montevideo, Uruguay).

Eighty-eight consumers participated in the trial, 61% of whom were male and 39% were female, ranging in age from 18 to 63 years. They were asked about their frequency of physical exercise and protein bar consumption, all ranges were included. Samples were served in pieces of approximately 10 g, on plastic plates coded using three-digit random numbers and presented to consumers in random order.

During testing, consumers had been provided with a label for each sample with nutritional information (as in Table 1) and indicating the claims that each bar fulfilled. Consumers were asked to indicate overall acceptability and purchase intent. Overall acceptability was evaluated on a nine points hedonic scale, from 1 ("Dislike extremely"), 5 ("Neither like nor dislike"), 9 ("Like extremely"). Purchase intent was measured on a seven-point scale, 1 ("Definitely would not buy"), 7 ("Definitely would buy"). Additionally, consumers answered CATA questions ("Check All That Apply"), checking all the terms that they considered described each sample. There were 13 CATA questions (detailed in Figure 1), most regarding textural and mouthfeel attributes. However, two of them were in reference to perceived nutritional qualities ("Healthy" and "Nutritious"), whether those were perceived from tasting alone or the nutritional information attached to each sample.

2.4 Statistical analysis

All data is reported as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed on each assay, and differences between samples were determined by the Tukey test ($\alpha \leq 0.05$). Pearson correlation tests were conducted ($\alpha \leq 0.05$). Cochran's Q test was performed to determine significant differences between samples on the frequency of each CATA attribute ($p \leq 0.05$).

Table 2. Nutritional characterization of Futura 75 hemp seed.

Dietary fiber	35,85 ± 0,30^{e}
Fat	28,43 ± 0,55^{d}
Protein	22,23 ± 0,34^{c}
Moisture	2,93 ± 0,20^{a}
Ash	5,63 ± 0,04^{b}
Carbohydrate*	5,06 ± 0,21^{b}

Means with a different letter are significantly different ($p < 0{,}05$). *Carbohydrate content was calculated by difference.

3 Results

3.1 Nutritional value of hemp seed

3.1.1 Nutritional characterization

Fat, protein, total dietary fiber, moisture, ash and carbohydrate content of whole Futura 75 hemp seed are shown in Table 2, as a percentage of total weight. The seeds most abundant macronutrient was dietary fiber, followed by fat, protein and lastly, carbohydrates. Significant differences were found between all macronutrients ($p < 0{,}05$).

3.1.2 Amino acid profile and protein nutritional quality

The amino acid profile of Futura 75 seeds used in the formulation of the snack bars is shown in Table 3, as grams of amino acid / 100 grams of protein. Results showed high levels of glutamine, having the highest concentration of all amino acids by a significant difference ($p < 0{,}05$). Additionally, tests revealed high contents of arginine, asparagine (aspartic acid) and leucine. Furthermore, the least abundant amino acids were cysteine, methionine, and histidine.

All essential amino acids were found.

Protein nutritional quality, shown in Table 4, depends on the aforementioned amino acid profile. Different indexes and parameters were measured relating to potential health benefits of the consumer, protein efficiency, biological value, and satiety indexes. Calculations showed promising results, especially regarding biological value, one of the satiety indexes (SAT2), the Lysine/Arginine ratio, BCAA content, Fischeŕs ratio, and all three protein efficiency ratios.

3.1.3 Fatty acid profile

Table 5 shows the seedś fatty acid profile, along with the sum of saturated, monounsaturated, polyunsaturated and unsaturated fatty acids respectively. In addition, the $\omega 6/\omega 3$ ratio was calculated to provide an insight into the potential health benefits of the seed. It was found that the predominant fatty acid was Linoleic Acid ($\omega 6$) with more than 50% of the total. However, because the levels of α Linolenic acid were also high, the $\omega 6/\omega 3$ ratio maintained a low value (4:1)

Table 3. Amino acid profile of Futura 75 hemp seed.

Amino acid	g/100g of protein
Asparagine	12,18 ± 0,26[i]
Threonine	4,09 ± 0,01[c,d]
Serine	5,86 ± 0,02[g]
Glutamine	18,73 ± 0,00[k]
Proline	4,64 ± 0,02[e]
Glycine	5,14 ± 0,13[f]
Alanine	5,23 ± 0,19[f]
Cysteine	1,80 ± 0,03[a]
Valine	5,59 ± 0,06[f,g]
Methionine	2,25 ± 0,03[a]
Isoleucine	4,48 ± 0,03[d,e]
Leucine	7,75 ± 0,16[h]
Tyrosine	3,93 ± 0,10[c]
Phenylalanine	5,43 ± 0,10[f,g]
Histidine	2,86 ± 0,06[b]
Lysine	4,02 ± 0,16[c,d]
Arginine	13,66 ± 0,16[j]
∑ EAA	36,47 ± 0,61
∑NEAA	68,84 ± 2,41

Means with a different letter are significantly different ($p < 0,05$). Σ EAA: sum of all essential amino acids. Σ NEAA: sum of all non-essential amino acids.

3.3 Sensory analysis

Table 6 details the results of acceptability and purchase intent of consumers for each sample, along with its most distinctive attributes. As it can be seen, the sample that reached higher acceptability (with significant differences with respect to samples S1 and S3) was Sample 2, containing 20% hemp seed, and "High Protein"-"High Fiber"-"Source of omega 3"/ "High Protein"-"Source of fiber"-"Source of omega 3" according to EU and Mercosur (local) regulations respectively. Additionally, the most frequent attributes for Sample 2 were "Moist" and "Tasty", whilst it had a significant minor frequency of the attribute "Off-flavor" than Sample 1, with the lowest acceptability and purchase intent, containing 40% hemp.

4 Discussion

4.1 Nutritional value of hemp seed

4.1.1 Nutritional characterization

Percentages of the major components, dietary fiber, fat and protein were within the ranges found in literature (Callaway, 2004; Leonard *et al.,* 2020; Vonapartis *et al.,* 2015), which reported (25%–35%), (30%–35%) and (25–30%) for fiber, fat and protein, respectively.

Therefore, hemp seed can be considered a food matrix with a high percentage of dietary fiber, composed of approximately 45%, 30% and 20% cellulose, lignin and hemicellulose, respectively (Vonapartis *et al.,* 2015). Fiber in hemp is composed in an 80:20 ratio between insoluble and soluble dietary fiber (Callaway, 2004). Approximately 70% of its dietary fiber is present in the seed's hull. Total dietary fiber ranges from 25–35% to 5–10% for dehulled seed (Leonard *et al.,* 2020). Additionally regarding hemp hull, most polyphenols, especially flavonoids, are found on this fraction of the seed (Siano *et al.,* 2018). Despite the above, hemp hulls are generally discarded prior to production. In response to trends and consumer awareness (Boukid *et al.,* 2022) on the impact of dietary fiber on digestive health, this study utilized whole hemp seeds to preserve the beneficial properties of the high-fiber hull.

4.1.2 Amino acid profile and protein nutritional quality

The amino acid profile of Futura 75 (Table 3) hemp seed consistent with previous research on different varieties (Callaway, 2004; Wang *et al.,* 2008).

In regard to protein quality, and with respect to essential amino acids, %EAAi, with values from 80% (shown in Table 4) indicates that the protein is of good quality (Mir *et al.,* 2019). Values found were higher than those for most cereals, as well as for flax, wheat and bran (Mir *et al.,* 2019; Oser, 1959; Sosulki *et al.,* 1973). In addition, protein sources such as soy, beef, casein and quinoa are found in similar ranges (Mir *et al.,* 2019; Oser, 1959; Sosulki *et al.,* 1973). Biological value (BV) is a function of EAAI and is a measure of the percentage of protein that is actually incorporated into proteins in the human body (Oser, 1959) Hemp seeds used in the study exhibited a biological value that corresponded to a good nutritional quality similar to that of soybean and with higher numbers than corn, other oilseeds such as canola (rapeseed) and almost double to that of wheat flour (Oser, 1959; Steve, 2012).

Additionally, the Lys/Arg ratio found in the seed was lower, than for conventional and highly consumed protein sources such as milk (2.23), egg (1.05) and soybean (0.73) (Iriondo-Dehond *et al.,* 2022). The above finding has relevance because the ratio of lysine to arginine is used as an index to evaluate the cholesterolemic and atherogenic effects of proteins, with a lower ratio indicating lower effects (Mir *et al.,* 2019). Furthermore, the sum of the amino acids arginine, histidine and glutamine is known to have a strong influence on the immune functions of the human body, and values for the seed were higher than for soy (28.46 g/100 g of protein), milk (27.84 g/100 g of protein) and egg (21.38 g/100 g of protein) (Iriondo-Dehond *et al.,* 2022).

Excessive caloric intake, coupled with a sedentary lifestyle, are substantial contributors to overweight and obesity. Therefore, it is relevant to consider the satiety levels that a potential functional food ingredient, in this case, hemp seed can achieve. Protein decreases calorie intake more than carbohydrates and fats and studies suggest that the satiating effects of proteins and differences between them are reflected in their amino acid composition. Results for the seed (SAT1 & SAT2) are shown in Table 4. SAT2 (8.02 g/100 g protein) proved to be higher than for soybean (5.14 g/100 g protein), milk (5.41 g/100 g protein) and egg (6.02 g/100 g protein) (Iriondo-Dehond *et al.,* 2022). The other satiety parameter, SAT1, was found to be lower than for milk (27.03 g/100 g protein) and egg (25.81 g/100 g protein), but higher than for soybeans (23.62 g/100 g protein) (Iriondo-Dehond *et al.,* 2022).

Regarding the Protein Efficiency Ratio (PER), values lower than 1.5 indicate low quality proteins and higher than 2.0

Table 4. Protein nutritional quality of Futura 75 hemp seed.

SAT1	SAT2	PER1	PER2	PER3	%EAAi	BV	BCAA	Fischer Ratio	Lys/Arg	Arg + His + Glu
24,27 ± 0,45	8,02 ± 0,10	3,07 ± 0,07	3,46 ± 0,08	2,10 ± 0,06	81,08 ± 1,35	76,67 ± 1,47	17,82 ± 0,25	1,90 ± 0,01	0,29 ± 0,01	35,25 ± 0,22

Satiety indicators (SAT), protein efficiency ratio (PER), essential amino acid index (%EAAi), biological value (BV), branched-chained amino acids (BCAA), lysine (Lys), arginine (Arg), histidine (His), glutamine (Glu).

Table 5. Fatty acid profile of Futura 75 hemp seed.

Fatty acid	Percentage of total (%)
C16:0 – Palmitic acid	$7{,}06 \pm 0{,}00^{e}$
C16:1n9 – Palmitoleic acid	$0{,}00 \pm 0{,}00^{a}$
C18:0 – Stearic acid	$3{,}06 \pm 0{,}08^{d}$
C18:1n9 – Oleic acid	$15{,}68 \pm 0{,}05^{g}$
C18:2n6 – Linoleic acid	$57{,}04 \pm 0{,}28^{h}$
C20:0 – Arachidic acid	$0{,}81 \pm 0{,}13^{b}$
C18:3n6 – γ Linolenic acid	$2{,}02 \pm 0{,}01^{c}$
C18:3n3 – α Linolenic acid	$13{,}84 \pm 0{,}26^{f}$
C20:1n9 – Eicosenoic acid	$0{,}50 \pm 0{,}03^{ab}$
Σ SFA	10,92 ± 0,21
Σ MUFA	16,18 ± 0,08
Σ PUFA	73,40 ± 0,58
Σ UFA	89,58 ± 0,63
Ratio $\omega6/\omega3$	4,10 ± 0,02

Means with a different letter are significantly different ($p < 0{,}05$). Σ SFA: the sum of all saturated fatty acids. Σ MUFA: the sum of all monounsaturated fatty acids. Σ PUFA: the sum of all polyunsaturated fatty acids. Σ UFA: the sum of all unsaturated fatty acids.

indicate high quality proteins (Mir *et al.*, 2019). The 3 different methods for PER calculation all shown hemp seed values higher than the standard of 2.0; therefore, hemp protein can be considered of high quality. In fact, PER1, PER2 and PER3 values remained in similar ranges to those of milk, egg and soy (Iriondo-Dehond *et al.*, 2022).

As for branched-chain amino acids (BCAA), hemp protein has values similar to those of soy (17.41 g/100 g of protein), milk (19.46 g/100 g of protein) and egg (19.48 g/100 g of protein) (Iriondo-Dehond *et al.*, 2022). It has been suggested in multiple studies that BCAAs are strongly linked to the stimulation of muscle synthesis and muscle recovery after physical exercise (Shimomura *et al.*, 2006).

Finally, Fischer's ratio (BCAA/AAA) for hemp seed gave results similar to those for egg (1.84), soy (1.64) and milk (1.95) (Iriondo-Dehond *et al.*, 2022). Reductions in Fischer's ratio are associated with an increase in the severity of liver failure and liver metabolism (Mohan *et al.*, 2020).

4.1.3 Fatty acid profile

Results for each individual fatty acid were as expected and reported by multiple previous research with different varieties of *Cannabis Sativa L* (Siano *et al.*, 2018, Pojić *et al.*, 2014).

The importance and positive impact of αLinolenic acid on human health, especially cardiovascular health, is already well known (De Lorgeril *et al.*, 1994), and it resulted within the expected range of 10–15% (Siano *et al.*, 2018, Pojić *et al.*, 2014). Presence of ΓLinolenic acid (GLA) also remained within expected ranges. Sources of GLA in nature are limited, and the fatty acid is a metabolic intermediate that leads to anti-inflammatory eicosanoids, attributing anti-cancer, vasodilatory and cholesterol-lowering effects (Saini *et al.*, 2018).

The sum of polyunsaturated fatty acids, above 70%, resulted in the expected range (Siano *et al.*, 2018).

Diets with a high ratio of $\omega6/\omega3$ increases the risk of obesity, and has an impact on cardiovascular and other chronic diseases (Simopoulos, 2008). The low ratio of $\omega6/\omega3$ found on the seed (4:1) is considered important for maintaining blood lipid levels and preventing high cholesterol levels (Simopoulos, 2002). The above indicates a positive nutritional profile, both for the whole hemp seed and for the oil extracted from it. The values are lower, *i.e.*, more desirable compared to $\omega6/\omega3$ profiles such as olive, sunflower or soybean (US National Nutrient Database, 2021).

Lastly, percentages of total saturated and unsaturated fatty acids were also consistent with previous studies on other hemp varieties (Siano *et al.*, 2018, Pojić *et al.*, 2014)

4.3 Sensory analysis

The level of acceptance by consumers is a vital parameter for assessing a newly developed food product. Acceptability of samples S2 and S3 (Table 6), reached values above the acceptable levels on a hedonic scale from 1 to 9, since acceptability values from 6.0 can be considered as a quality or commercial limit (Munoz, 2013).

As for purchase intent, Sample 1 had the lowest values despite having the higher nutritional claims according to Mercosur (local) regulation. From a scientific perspective, this data suggests that nutritional claims alone are not enough to drive purchase intent. Sensory attributes, such as taste, texture, and appearance, play a crucial role in consumer decision-making when it comes to food products, as will be discussed further in the study.

Correlations between protein bar consumption, physical activity frequency, purchase intent and overall acceptability of consumers were all significant ($\alpha = 0{,}05$) and positive, and the strongest of them all was found between overall acceptability and purchase intent, as it was widely anticipated. The observed positive correlation between physical exercise frequency and the overall acceptability – purchase intent of snack bars with the claims "high in protein" and "high in fiber" as shown in this study, suggests that such products may be well-suited for the sports nutrition market, and is supported by the trends detailed by Boukid *et al.* (2022). In fact, previous research shows that

Table 6. Acceptability, purchase intent and main distinctive attributes for the three snack bar samples.

	Acceptability	Purchase intent	Moist (frequency)	Tasty (frequency)	Off-flavor (frequency)
Sample 1 (S1)	$5,8^{a} \pm 2,1$	$4,3^{a} \pm 1,6$	32^{a}	28^{a}	28^{b}
Sample 2 (S2)	$6,9^{b} \pm 1,6$	$5,2^{b} \pm 1,5$	51^{b}	44^{b}	15^{a}
Sample 3 (S3)	$6,2^{a} \pm 2,0$	$4,6^{a,b} \pm 1,9$	36^{a}	$31^{a,b}$	$20^{a,b}$

Means with a different letter within the same columns are significantly different ($p < 0{,}05$).

"high protein" claim increases consumer interest, especially among exercisers (Salazar *et al.,* 2019). However, targeting the sports nutrition market is not the only implication of including high protein and fiber claims in snack bars, as package attributes such as nutritional claims have been shown to influence the purchase intent of snack bars for all types of consumers, regardless of their exercise habits, as demonstrated by Pinto *et al*. (2017).

After CATA analysis, it was possible to detail the main attributes of each sample (Table 6), as well as to see the influence of those attributes on their increase or decrease in total acceptability. For the attribute "Tasty", a significant difference was seen between S2 and S1, samples with the higher and lower acceptability, respectively. S3 remained between the two, with no significant differences. In turn, for the "Moist" attribute, there was a significant difference for consumers between S2 and the other two samples. The above is of great importance because these two attributes are the ones that had a significantly positive impact ($p < 0.05$) on consumer acceptability.

Lastly, "Off-flavor" was noteworthy for sample S1. "Off-flavor" refers to objectionable flavors, including perceived undesirable tastes, odors, and other sensations such as astringency (Wang *et al.,* 2022). In fact, this attribute was the one with the higher negative impact in consumer acceptability, the only attribute with a significantly negative impact ($p < 0{,}05$). This could be explained by two factors, which may act separately or as a whole:

- S1 had a lower percentage of dates than the other two samples. The lack of sweetness provided by this fruit might have been missing if the consumer was looking for the typical sweetness of a snack bar. In fact, Byrd-Bredbenner *et al.* (2012), found that for snack vending machines located in buildings with the greatest student traffic flow on 11 U.S. education campuses, almost half of the snacks were "high in sugar", and that "the most common snacks for sale in vending machines were salty snacks and sweets (*i.e.*, candy and candy bars)";
- the other aspect to consider is that S1 was the one with higher hemp seed percentage on formulation. Given that the seed is proposed in this study as a new ingredient in functional foods, most if not all consumers tasted the seed for the first time at the sensory analysis, so that could be a reason why they deemed S1 with an "off-flavor". Wang *et al.* (2022) determined which volatile and nonvolatile compounds were responsible for off-flavors for a wide variety of plant-based protein sources, amongst which hemp was evaluated, and authors found 16 hemp seed compounds that could negatively impact on consumers taste, most related with a bitter after-taste. In the study for baked products by Mikulec *et al.* (2019), a significant decrease in consumer acceptability was already found with hemp percentages of 50% compared to those of 15% and 30%, whilst reporting an increasing bitter taste. Based on the information presented above, it is noteworthy that there seems to exist a threshold for the maximum amount of hemp that can be incorporated into a functional food formulation, for sensory reasons, mainly the bitter after-taste that the seed may attribute to the final product.

The most repeated terms in the sum of the three samples were "Nutritious" and "Healthy" respectively. This is likely due to the nutritional information attached to the samples. The impact of this information remains to be conclusively evaluated in future studies.

5 Conclusion

Based on the findings of this study, it is suggested that hemp seed may have potential as a functional food ingredient to meet the demand for plant-based alternatives, providing high-quality protein with a remarkable amino acid profile, and potentially promoting digestive health due to its high fiber percentage. Furthermore, the seed's high lipid content is characterized by a low $\omega 6/\omega 3$ ratio (4:1), which is considered to be critically important in regulating blood lipid levels and preventing elevated cholesterol levels. This helps improve the health of consumers and prevent non-communicable diseases, which is one of the primary objectives in developing functional foods.

Two of the products formulated with hemp seed met the sensory acceptability criteria or commercial standards, both containing 20% hemp. Additionally, a positive correlation was observed between physical exercise and the overall acceptability – purchase intent of the samples. Nevertheless, it is important to note that there appears to be an upper limit to the amount of hemp seed that can be added to a product formulation due to sensory reasons. Specifically, this study found that inclusion rates of up to 40% in a snack bar formulation had a negative effect on consumer acceptability and purchase intent. Therefore, careful consideration should be given to the optimal amount of hemp seed to be included in a product formulation to balance the nutritional benefits with the sensory properties required for consumer acceptance. Further studies are required to determine the optimal concentration of hemp seed in various food products.

Conflicts of interest

All authors declare that they have no conflicts of interest.

References

Anderson JW, Baird P, DaviJ Jr, RH *et al.* 2009. Health benefits of dietary fiber. *Nutr Rev* 67 (4): 188–205.

Barkholt V, Jensen AL. 1989. Amino acid analysis: determination of cysteine plus half-cystine in proteins after hydrochloric acid hydrolysis with a disulfide compound as additive. *Anal Biochem* 177 (2): 318–322.

Boukid F, Klerks M, Pellegrini N, *et al.* 2022. Current and emerging trends in cereal snack bars: implications for new product development. *Int J Food Sci Nutr* 73 (5): 610–629.

Byrd-Bredbenner C, Johnson M, Quick VM, *et al.* 2012. Sweet and salty. An assessment of the snacks and beverages sold in vending machines on US post-secondary institution campuses. *Appetite* 58 (3): 1143–1151.

Callaway JC. 2004. Hempseed as a nutritional resource: an overview. *Euphytica* 140: 65–72.

Coello KE, Frias J, Martínez-Villaluenga C, Cartea ME, Velasco P, Penas E. 2022. Manufacture of healthy snack bars supplemented with moringa sprout powder. *LWT* 154: 112828.

De Lorgeril M, Renaud S, Salen P, *et al.* 1994. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 343 (8911): 1454–1459.

Greco E, Winquist A, Lee TJ, *et al.* 2017. The role of source of protein in regulation of food intake, satiety, body weight and body composition. *J Nutr Health Food Eng* 6 (6): 00223.

Irakli M, Tsaliki E, Kalivas A, Kleisiaris F, Sarrou E, Cook CM. 2019. Effect of Genotype and Growing year on the nutritional, phytochemical, and antioxidant properties of industrial hemp (Cannabis sativa L.) seeds. *Antioxidants* 8 (10): 491.

Iriondo-DeHond A, del Pilar Salazar M,Domènech LG, Montoya JEZ, del Castillo MD. 2022. Coffee Proteins: functional food ingredients with molecular effects for sustainable health. *Molecular Mechanisms Funct Food* 382–403.

Lachenmeier DW, Kroener L, Musshoff F, Madea B. 2004. Determination of cannabinoids in hemp food products by use of headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Anal Bioanal Chem* 378: 183–189.

Lattimer JM, Haub MD. 2010. Effects of dietary fiber and its components on metabolic health. *Nutrients* 2 (12): 1266–1289.

Leonard W, Zhang P, Ying D, Fang Z. 2020. Hempseed in food industry: nutritional value, health benefits, and industrial applications. *Compr Rev Food Sci Food Saf* 19 (1): 282–308.

Mikulec A, Kowalski S, Sabat R, Skoczylas Ł, Tabaszewska M, Wywrocka-Gurgul A. 2019. Hemp flour as a valuable component for enriching physicochemical and antioxidant properties of wheat bread. *LWT* 102: 164–172.

Mir NA, Riar CS, Singh S. 2019. Effect of pH and holding time on the characteristics of protein isolates from Chenopodium seeds and study of their amino acid profile and scoring. *Food Chem* 272: 165–173.

Mohan N, Mellem JJ. 2020. Functional properties of the protein isolates of hyacinth bean [Lablab purpureus (L.) Sweet]: an effect of the used procedures. *Lwt* 129: 109572.

Munoz AM, ed. 2013. Sensory evaluation in quality control. Springer Science & Business Media; 2013 Nov 11.

Oser BL. 1959. An integrated essential amino acid index for predicting the biological value of proteins. *Protein Amino Acid Nutr* 281.

Paquot C. 2013. Standard methods for the analysis of oils, fats and derivatives. *Elsevier. IUPAC* 2. 301–2.304.

Pinto VRA, de Oliveira Freitas TB, de Souza Dantas MI, *et al.* 2017. Influence of package and health-related claims on perception and sensory acceptability of snack bars. *Food Res Int* 101: 103–113.

Pojić M, Mišan A, Sakač M, *et al.* 2014. Characterization of byproducts originating from hemp oil processing. *J Agric Food Chem* 62 (51): 12436–12442.

Saini RK, Keum YS. 2018. Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance—a review. *Life Sci* 203: 255–267.

Salazar NA, Fiszman S, Orrego CE, Tarrega A. 2019. Evaluation of some ingredients and energy content on front-of-pack cereal bar labeling as drivers of choice and perception of healthiness: a case study with exercisers. *J Food Sci* 84 (8): 2269–2277.

Schuster R. 1988. Determination of amino acids in biological, pharmaceutical, plant and food samples by automated precolumn derivatization and high-performance liquid chromatography. *J Chromatogr B: Biomed Sci Appl* 431: 271–284.

Shimomura Y, Yamamoto Y, Bajotto G, *et al.* 2006. Nutraceutical effects of branched-chain amino acids on skeletal muscle. *J Nutr* 136 (2): 529S–532S.

Siano F, Moccia S, Picariello G, *et al.* 2018. Comparative study of chemical, biochemical characteristic and ATR-FTIR analysis of seeds, oil and flour of the edible Fedora cultivar hemp (*Cannabis sativa L.*). *Molecules* 24 (1): 83.

Simopoulos AP. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother* 56 (8): 365–379.

Simopoulos AP. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med* 233 (6): 674–688.

Sosulski FW, Sarwar G. 1973. Amino acid composition of oilseed meals and protein isolates. *Can Inst Food Sci Technol J* 6 (1): 1–5.

Steve IO. 2012. Influence of germination and fermentation on chemical composition, protein quality and physical properties of wheat flour (Triticum aestivum). *J Cereals Oil Seeds* 3 (3): 35–47.

Teterycz D, Sobota A, Przygodzka D, Łysakowska P. 2021. Hemp seed (*Cannabis sativa L.*) enriched pasta: Physicochemical properties and quality evaluation. *Plos one* 16 (3): e0248790.

Trovato E, Arena K, La Tella R, *et al.* 2023. Hemp seed-based food products as functional foods: a comprehensive characterization of secondary metabolites using liquid and gas chromatography methods. *J Food Compos Anal* 117: 105151.

US National Nutrient Database, Release 28, *United States Department of Agriculture*.xx xxxxxx

Volk C, Brandsch C, Schlegelmilch U, *et al.* 2020. Postprandial metabolic response to rapeseed protein in healthy subjects. *Nutrients* 12 (8): 2270.

Vonapartis E, Aubin MP, Seguin P, Mustafa AF, Charron JB. 2015. Seed composition of ten industrial hemp cultivars approved for production in Canada. *J Food Compos Anal* 39: 8–12.

Wang XS, Tang CH, Yang XQ, Gao WR. 2008. Characterization, amino acid composition and *in vitro* digestibility of hemp (*Cannabis sativa L.*) proteins. *Food Chem* 107 (1): 11–18.

Wang Y, Tuccillo F, Lampi AM, *et al.* 2022. Flavor challenges in extruded plant-based meat alternatives: a review. *Compr Rev Food Sci Food Saf* 21 (3): 2898–2929.

Cite this article as: Presa-Lombardi J, García F, Gutierrez-Barrutia MB, Cozzano S. 2023. Hemp seed's (*Cannabis Sativa L*) nutritional potential for the development of snack functional foods. *OCL* 30: 24.