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يسر **معامل التأثير والاستشهادات المرجعية للمجلات العلمية العربية (ارسيف – ARCIF)**، أحد مبادرات قاعدة بيانات **"معرفة"** للإنتاج والمحتوى العلمي، إعلامكم بأنه قد **أطلق التقرير السنوي التاسع للمجلات للعام 2024.**

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Production and Evaluation of High Branched-chain Amino Acids (BCAAs) Pasta for Liver Cirrhosis Patients

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Abstract

This study aims to produce and evaluate high branched-chain amino acids (BCAAs) pasta as a functional food that contributes to reducing liver cirrhosis. The chemical composition of the raw materials, physicochemical and sensory properties of the pasta samples, were studied. The best pasta sample was selected based on sensory evaluation. The results indicated that the highest protein content was obtained from soy protein isolate (SPI) (88.04%), followed skimmed milk powder (SMP) (36.18%), compared to 28.80%, 35.34%, 21.54% recorded by legume seeds (lentils, lupine, and chickpeas, respectively).

Keywords: Liver cirrhosis, BCAA, pasta, legumes, Fisher ratio.

ملخص:

العنوان : إنتاج وتقييم مكرونة غنية بالأحماض الأمينية المتشعبة السلسلة (BCAAs) لمرضي تليف الكبد

المؤلفون : أسامة السيد مصطفى ، أماني أحمد عبد العزيز ، مها مهدي عدلي

تهدف هذه الدراسة إلى إنتاج وتقييم مكرونة عالية المحتوى من الأحماض الأمينية المتفرعة السلسلة (BCAAs) كغذاء وظيفي يساهم في تقليل تليف الكبد. تم دراسة التركيب الكيميائي للمواد الخام، وكذلك الخصائص الفيزيوكيميائية والحسية لعينات المكرونة. تم اختيار أفضل عينة بناءً على التقييم الحسي. أوضحت النتائج إلى أن أعلى قيمة لمحتوى البروتين متحصل عليه من بروتين الصويا المعزول (SPI) (88.04%)، تليها مسحوق الحليب خالي الدسم (SMP) 36.18% بواسطة ، مقارنة بـ 28.80%، 35.34% و21.54% التي سجلتها بذور البقوليات (العدس، الترمس، والحمص على التوالي).

الكلمات الدالة : تليف الكبد، الأحماض الأمينية المتفرعة السلسلة (BCAA) ، مكرونة ، البقوليات، نسبة فيشر

Introduction

The liver is the largest solid organ in the human body, it performs over 500 essential functions, including protein synthesis, detoxification, and amino acid metabolism. Factors such as viral hepatitis (B and C), metabolic disorders, autoimmune conditions, congenital anomalies, alcohol consumption, medical drugs, and nonalcoholic fatty liver disease (NAFLD) can negatively impact the liver, potentially leading to liver cirrhosis **(Matyas *et al*., 2021; Faccioli *et al*., 2022).**

Liver cirrhosis a common disease that alters liver function and structure, is the final stage of chronic liver diseases. It has two phases: compensated, which is often asymptomatic or has nonspecific symptoms, and decompensated, which involves complications like ascites, hepatic encephalopathy, and variceal bleeding due to portal hypertension **(Toshikuni *et al*., 2014; D'Amico and Malizia 2012)**.

Long-term consumption of medications for treating liver cirrhosis is controversial and causes adverse reactions. Recent research has highlighted the importance of functional foods that exert protective and therapeutic effects based on their antioxidant, anti-inflammatory, and detoxifying properties on the liver. Additionally, some dietary supplements have mechanisms of action that make them beneficial for liver diseases **(Fallowfield *et al*., 2021; Janugade *et al*., 2024).**

Branched-chain amino acids (BCAAs), including valine, leucine, and isoleucine, are crucial for protein structure and play significant roles in liver disease mechanisms. They make up 20-25% of dietary protein and 35% of essential amino acids in mammals, who cannot synthesize them and must obtain them through diet and are metabolized outside the liver, thereby imposing no additional burden on the compromised organ, Since, it has been described for liver cirrhotic people **(Blair *et al*., 2021)**. Low BCAAs and high aromatic amino acids (AAAs) are shared in typical abnormalities in the blood of patients with liver cirrhosis, which play a role in the pathogenesis of liver encephalopathy and muscle loss **(Varshney and Saini, 2020)**.

The meal components are considered one of the most important factors that determine its role as a functional food, so it is very important to the meal's abundant amounts of content BCAAs, which are considered the backbone of the therapeutic meal for patients with liver cirrhosis, which was confirmed by **Takeda *et al.* (2021)**, they stated that treatment with BCAAs attenuated hepatic inflammation and fibrosis and improved skeletal muscle atrophy and strength in rats. and reduced intramuscular myostatin and pro-inflammatory cytokine levels. **Hiraoka *et al.* (2017)** reported that patients with hepatic cirrhosis consumed simple addition of carbohydrate and protein-rich in BCAAs evening snack may help in nitrogen balance, improve muscle cramps and prevent muscle breakdown by supplying the body with overnight carbohydrate energy, and preventing gluconeogenesis.

Plant-based diets represent a valid strategy to improve human health and increase food sustainability. **(Cioffi *et al.*, 2024)**. Legume-based products, which are excellent sources of proteins and fibers, are also affordable and readily available. These legumes are often consumed after processing, which improves their palatability and nutrient bioavailability by inactivating growth inhibitors and trypsin inhibitors **(Gu *et al.*, 2023).** This can help consumers adopt healthier dietary patterns **(Cioffi *et al.*, 2024).** The health benefits of legumes, such as soybeans, lupine, chickpeas, and lentils **(Bouchenak and Lamri-Senhadji, 2013)**, are largely due to their rich nutritional composition with phenolic compounds, phytates and antioxidants **(Barman *et al.*, 2018; Rachwa-Rosiak *et al.*, 2015; Singh, 2017)**. Ethanol extracts of legumes have shown potential as supplements for metabolic syndromes, with their efficacy supporting a multi-targeting approach to mitigate non-alcoholic fatty liver disease (NAFLD) **(Koh *et al.*, 2020)**. Additionally, lupin protein hydrolysates have demonstrated lipid-lowering, antioxidant, and anti-inflammatory effects. Notably, this is the first study to show that lupine supplementation can reduce the development of a steatotic liver caused by a Western diet and decrease abdominal obesity **(Santos *et al*., 2021)**. Plant protein positively affected the components of the intestinal microbiome **(Merli *et al*., 2016)**.

Furthermore, research indicates that legumes, being a primary source of higher amounts of branched chain amino acids, starches, and non-starchy carbohydrates antioxidants, minerals, and vitamins, higher amount of branched chain amino acids, starches, and non-starchy carbohydratesmay help mitigate the risk of liver cirrhosis **(Amarowicz and Pegg, 2008; Iqbal *et al*., 2022)**

Pasta is rapidly becoming one of the most beloved foods globally, second only to bread. Its rise in popularity can be attributed to its convenience, affordability, appealing taste and texture, long shelf life, easy preparation, and overall deliciousness **(Veena and Shivaleela, 2019).**

In cirrhotic patients, the provision of amino acids to the muscles is deranged. Taken together, BCAAs are single ingredients avoid proteolysis and lipolysis, motivate albumin synthesis, maintain nitrogen balance, stimulate favorable effect on muscle mass and exhibit an improvement in live encephalopathy, their general condition, and life quality in these patients **(Ichikawa *et al*., 2013)**.

Recent studies indicate that patients with cirrhosis are at high risk for protein-calorie malnutrition due to altered metabolism. Current evidence has shifted from the old belief of protein restriction in these patients, now recommending a protein intake of 1.2 to 1.5 g/kg/day **(Iqbal *et al*., 2022)**. Additionally, European guidelines suggest prescribing 0.25 g/kg/day of branched-chain amino acids (BCAAs) for patients with advanced cirrhosis to improve event-free survival and quality of life **(Bischoff *et al*., 2020)**. Therefore, this study aimed to prepare pasta rich in BCAAs made from legumes (lentil, lupine, chickpea, and soy protein isolate) as a functional food to help improve liver function and reduce liver cirrhosis.

MATERIALS AND METHODS

Materials

Lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), lupines (*Lupinus* spp. L.), skimmed milk powder were purchased from the local market. Soy protein isolate was obtained from American FoodChem. Carboxymethyl cellulose (CMC) obtained from Sigma Aldrich. Other used chemicals were purchased from El-Gomhoria and El Shark El Aost Companies, Egypt.

Methods

Preparation of legumes (lentil, chickpea and lupine) flour

The legumes flour was prepared according to **Giami and Bekebain (1992)**. One kilogram of legume seeds was cleaned of dirt and other foreign materials such as stones and sticks, then soaked in 2L tap water for 12 hr. Seeds were ground by using a mixer (MIENTA super blender, Model BL -721) and dried in the cabinet dryer (120°C/90 min). During drying, the ground seeds were stirred at intervals of 30 minutes to ensure uniform drying. The ground seeds were sieved to pass through a 300 mesh sieve. The obtained flour was finally packaged in sealed polyethylene bags until used.

Preparation of pasta

Pasta dough was prepared from different portions of legumes flour as presented in Table 1, according to the procedure reported by **Collins and Pangloli (1997)** *on the basis of 600 g flour. All dry ingredients were combined and mixed to produce homogenized blend. Placed the mixture in a mixing bowl and kneaded until the dough formed. The dough was rounded (shaped into a ball), covered with plastic wrap, allowed to rest 30 min, manually kneaded 1min, divided into about 100-g portions and* *formed using pasta machine (Philips Pastamaker HR2357/05 Machine Corporation, Italy). The drying process of pasta was conducted according to* **Mostafa (2020)**, *formed pasta was exposed to* air-drying *at 23-25 °C for 4 h. A fan operated in the room to facilitate drying. The air-dried* Pasta was *transferred to a cabinet dehydrator and dried* to a moisture content *12 % at 70 °C. Thereafter, cooled at room temperature (25°C±2), placed in plastic bags, sealed the plastic bags and stored at 12-14°C until tested.*

Table 1. Pasta formulas

Ingredients (g)	Formulas		
	P1	P2	P3
Soy protein isolate	40	40	40
Skimmed milk	10	10	10
Lentil	30	10	10
Chickpea	10	30	10
Lupine	10	10	30
Carboxymethyl cellulose	2.50	2.50	2.50

Analytical methods

Chemical composition of raw materials and final products

Chemical analysis was performed for raw materials and final products. The moisture, protein, fat, crude fiber and ash of raw materials and the produced pasta were determined according to the **AOAC (2005)**. Total carbohydrates were calculated by difference.

Minerals determination

Mineral quantification was carried out by atomic absorption spectrophotomete (type AAnalyst 400, Perkin–Elmer, Waltham, MA, USA) after sample digestion with HCl as described by **Gupta *et al.* (2006)**.

Color determination

The color of samples was measured according to **Hunter (1958)** using a Hunter Lab colorimeter. Color parameters L*, a* and b* parameters were determined by a spectro-colorimeter

(Tristimulus Color Machine) with CIE lab color scale (Hunter, Lab Scan XE-Reston VA, USA) with the reflection mode. Instrument was standardized with black and white tile Hunter Lab Color Standard (LX No.16379), X = 72.26, Y = 81.94 and Z = 88.14 (L* = 92.46, a* = -0.86; b* = -0.16). The instrument (65°/0° geometry; D25 optical sensor; 10° observer). Color values were expressed as lightness to darkness for L*, redness to greenness for a* and yellowness to blueness for b* parameter.

Amino acids pattern

- Amino acids content of samples was determined according to the method described in **AOAC (2005)**.
- Protein Efficiency Ratio (PER) a = 0.456 + 0.454 (Leucine) - 0.047 (pro) and Protein Efficiency Ratio (PER) b = 0.498 + 0.454 (Leucine) - 0.105 (Tyrosine) were calculated **(Alsmeyer *et al.*, 1974)**.
- The biological value (B.V) was assayed according to the following equation which was recommended by **Eggum *et al.* (1979)** by the following equation: B.V.% = 39.55 + 8.89 × lysine (g/100g protein).
- The molar ratio of branched-chain amino acid (BCAA) residues (Leu, Ile and Val) to aromatic amino acid (AAA) residues (Tyr, Trp and Phe) is known as the Fischer ratio **(Wang *et al.*, 2024)**.

Cooking quality of pasta

- **Pasta cooking quality** was determined according to the approved method in **AACC (2000)**.
- **Optimum cooking time** was the time required for the opaque central core of the pasta to disappear when squeezed gently between two glass plates after cooking. About 25 g of pasta was cooked to optimum time in 300 mL tap water in a beaker, rinsed in cold water and drained for 15 min before being weighed. Percentage of increased

weight calculated as a cooking yield. Solids content in the cooking water was determined by drying at 105 °C overnight **(AACC, 2000)**.

- **Cooking loss** was expressed as a percentage between the solid weight and initial dry matter **(AACC, 2000)**.
- **Nitrogen loss** was determined according to the approved Kjeldahl method in American Association of Cereal Chemists **(AACC, 2000)** using a conversion factor of 5.7.

Texture profile analysis of pasta

The texture of pasta samples **(**hardness) was determined by Texture Profiles Analysis **(**TPA) CT3™ Texture Analyzer **(**Brookfield) according to **Boume (2002)**. The computer was set for Test works software and an appropriate test was selected for the TPA analysis: test speed 2.50mm/s, load cell 10 Kg in two cycles for cooked pasta and one cycle for uncooked pasta with 10 mm depth. The parameters like length, diameter, speed, the percent compression, and number of cycles were given as input data to the computer before staring the compression of the sample.

Sensory evaluation of pasta

Pasta products were cooked in boiling water without the addition of salt, drained and placed in warm conditions until testing. An 18-member semi-trained panel was asked to evaluate pasta products for overall quality in terms of color (20), taste (20), texture (20), appearance (20), flavor (20) and overall acceptability (100) **(Larmond, 1970)**.

Ethical Approval:

The laboratory procedures and animals were handled following the guidelines published by the Local Committee of the Faculty of Specific Education, South Valley University according to the Animal Ethical Guidelines Procedures Act with approval No (166180924).

Statistical analysis

The obtained results were statistically analyzed using the SPSS statistical package (Version 20) and analysis of variance (ANOVA). Duncan's multiple range test and least significant difference (LSD) were chosen to determine any significant difference among various treatments at p<0.05.

RESULTS AND DISCUSSION

Chemical composition of used raw materials

The approximate analysis of raw materials was estimated for moisture, ash, protein, fat, crude fiber and total carbohydrates, the obtained data are shown in Table 2. According to the obtained results, skimmed milk powder (SMP) contained 2.53% moisture, 7.34% ash, 36.18% protein, 1.02% fat and 52.93% total carbohydrates. Whereas, soy protein isolate recorded 5.40, 3.68, 88.04, 0.51, 0.33 and 2.04 for moisture, ash, protein, fat, fiber and total carbohydrates respectively. The obtained results are also in harmony with **Wróblewska *et al.* (2018)**, they reported that soy protein isolate (SPI) contained 85.00% protein, 3.00% fat, and 1.00% total carbohydrates. Also, **Soria-Hernández *et al.* (2020)** reported that SPI contained 91.82% protein 0.09% fat and 4.68% ash content. **Hameed *et al*. (2021)** stated that SMP contained 5.33% moisture, 38.69% crude protein, 1.32% lipids, 8.10% ash and 46.55% total carbohydrates. Our findings also in a harmony with **Barone *et al*. (2020)**, they find that SMP contained 3.99 g/100g moisture, 35.60 g/100g protein, 0.52 g/100g fat, 7.59 g/100g ash and 48.60 g/100g total carbohydrates.

Concerning the chemical composition of legume seeds, lentil seeds recorded 6.15% moisture, 2.96% ash, 28.80% protein, 0.61% fat 3.59% crude fiber, and 57.89% total carbohydrates. While, lupine seeds contained 6.68% moistutre, 4.74% ash, 35.34% protein, 6.94% fat, 8.35% crude fiber and 37.77% total carbohydrates. Also, chickpea recorded 4.33, 3.03, 21.54, 4.98, 1.72 and 64.40% of moisture, ash, protein, fat, crude fiber and total carbohydrates respectively. Therefore, the highest values of protein content of legumes were recorded by lupine followed by lentil then chickpea which recorded the lowest value of protein content. However, the chickpea seeds recorded the highest total carbohydrate value compared to other samples. With regard to the crude fiber, the highest values were obtained by lupine and lentil seeds respectively. These results were in agreement with **Zia-Ul-Haq *et al.* (2007)**, they studied the chemical analysis of different varieties of lentil and they found that they contained 28.80 to 30.60% of protein content. Another study was investigated by **Ahmed *et al.* (2016)** who reported that lentil flour contained 10.40% moisture, 25.80% protein, 1.10% fat, 2.70% ash and 60% total carbohydrates. Also, **Khan *et al.* (1995)** studied that approximate analysis of chickpea flour, and they stated that it contained 24.40% protein, 3.70% fat, 3.90% fiber, 3.20% ash and 47.40% carbohydrates. Thus, the chemical composition varies depending on growing conditions, year, and varieties and according to the study which investigated by **Li and Ganjyal (2017)**, lentils had higher protein content (25.8–28.6%), 2.0-305 ash, 8.30-10.0% moisture, 0.60-1.0% lipids and 43.5–50.0%. While, **Chung *et al*. (2008)** stated that lintel seeds contain a range of moisture from 8.60 to 8.80%, protein from 31.50 to 28.70%, fat from 2.40 to 2.30% and starch from 47.10 to 46.00%.

Table 2: Chemical composition of used raw materials

Samples	Chemical composition (% on w.w.b.)					
	Moisture	Ash	Protein	Fat	CF	TC
Lentil	6.15a±0.12	2.96d±0.06	28.80c±0.44	0.61d±0.02	3.59b±0.06	57.89b±1.07
Lupine	6.68a±0.17	4.74b±0.14	35.34b±0.29	6.94a±0.13	8.53a±0.22	37.77d±0.71
Chickpea	4.33c±0.12	3.03d±0.04	21.54d±0.31	4.98b±0.08	1.72c±0.04	64.40a±1.19
SMP	2.53d±0.03	7.34a±0.19	36.18b±0.47	1.02c±0.03	ND	52.93c±1.23
SPI	5.40b±0.16	3.68c±0.07	88.04a±0.72	0.51d±0.01	0.33d±0.01	2.04e±0.03
LSD at 0.05	0.581	0.336	2.584	0.214	0.443	1.840

Where: CF= crude fiber, TC= total carbohydrates, SMP= skimmed milk powder, SPI= soy protein isolate.

Minerals content of used raw materials

Table 3 presented the minerals content of raw materials, and from the obtained results it can be seen that, skimmed milk powder (SMP) recorded 0.57 mg/100g Fe, 1.07 mg/100g Zn, 1417.34 mg/100g K, 187.55 mg, and 369.21 mg/100g Na. While, soy protein isolate (SPI) recorded 7.52, 4.42, 859.95, 75.27 and 487.88 mg/100g for Fe, Zn, K, Mg and Na respectively. **Uddin *et al.* (2016)** studied the minerals content of SPI and they reported that it contained 22.19 mg/100g iron, 1081.84 mg/100g potassium, 1030.02 mg/100 sodium, 9.08 mg/100g zinc and 96.48 mg/100 magnesium. The significant effect of genotype, and soil minerals on the mineral content of soybean was shown by **Esper *et al.* (2021)**. On the other side, **Barone *et al*. (2020)**, reported that SMP contains 1743 mg/100g K, 107 mg/100g Mg, 371 mg/100g Na, 0.16 mg/100g Fe and 3.94 mg/100g Zn.

With regard to the minerals content (Fe, Zn, K, Mg and Na) of legume seeds, lentil seeds contained 3.72, 2.10, 743.50, 120.00 and 72.23 mg/100g, respectively. While the lupine contained 8.60, 3.07, 589.50, 437.00 and 199.94 mg/100g, respectively. Data also showed that chickpea recorded 6.43mg/100g Fe, 3.87 mg/100g Zn, 649.00 mg/100g K, 148.50 mg/100g Mg and 123.44 mg/100g Na. Concequently, the lupine seeds recorded the highest value of iron content followed by chickpeas. As for the zinc content of legume seeds the highest value was recorded by chickpeas in contrast to lentil seeds which showed the lowest content of Zn compared to chickpea and lupine. Meanwhile the lupine seeds recorded the highest value of Mg and Na compared to chickpea and lentil seeds. The Minerals content of two types of chickpea was previously estimated by **Wang *et al.* (2010)** and their results indicated that contained 994.50-1060.00 mg/100g potassium, 169.00-147.00 mg/100g magnesium, 4.59-5.50 mg/100g iron and 4.07-3.40 mg/100g zinc. While **Çoban *et al.* (2021)** reported that lupine contained iron 0.00–149.23 mg/kg, magnesium 34.07–2002.53 mg/kg, potassium 2463.21–6779.90 mg/kg, and sodium 91.69–8532.82 mg/kg. The difference in mineral content can be due to the climate or geographical condition of the area where these crops are grown. The minerals content of lentil flour was investigated by **Atudorei *et al*. (2021)**

and they stated that it contained 26.08 mg/100g sodium, 122.35 mg/100g magnesium, 2.62 mg/100g iron, 3.07 and mg/100g zinc.

Table 3: Minerals content of used raw materials

Samples	Minerals content (mg/100g)				
	Iron	Zinc	Potassium	Magnesium	Sodium
Lentil	3.72d±0.04	2.10d±0.06	743.50c±15.21	120.00d±3.12	72.23e±3.00
Lupine	8.60a±0.33	3.07c±0.04	589.50e±18.18	437.00a±13.36	199.94c±7.25
Chickpea	6.43c±0.26	3.87b±0.09	649.00d±22.37	148.50c±9.14	123.44d±5.37
SMP	0.57e±0.03	1.07e±0.03	1417.34a±38.16	178.55b±11.02	369.21b±19.20
SPI	7.52b±0.38	4.42a±0.08	859.95b±29.15	75.27e±4.17	487.88a±13.79
LSD at 0.05	0.711	0.14	41.765	21.793	28.604

SMP= skimmed milk powder, SPI= soy protein isolate.

Amino acids profile and protein quality of used raw materials

The amino acids profile of used raw materials were estimated as g/100 sample and the obtained data are shown in Table 4. Concerning the essential amino acids (EAAs) content, skimmed milk powder (SMP) showed higher values of branched-chain amino acids (4.05, 2.04 and 2.01 g/100g sample) for leucine, isoleucine and valine respectively. These results are in agreement with **Magan *et al.* (2019)**, they mentioned that the highest values of EAAs of SMP were recorded by leucine (93.50 g/kg protein), lysine (72.60 g/kg protein) and valine (55.30 g/kg protein). Also, they found that cysteine recorded the lowest value of total EAAs. Others, **(Hameed *et al.*, 2021)** studied the effect of different drying methods on amino acids content of SMP and they found that the EAAs ranged between 2.53-3.28 g/100g sample (leucine), 2.36-3.04 g/100g sample (lysine) and 1.72-2.25 g/100g sample (valine).

Also, the highest values of EAAs of soy protein isolate (SPI) was recorded by leucine, isoleucine, lysine, phenylalanine and valine (8.13, 5.41, 5.40, 4.50 and 4.47 mg/ 100g sample, respectively). The obtained results are in a harmony with **Wróblewska *et al.* (2018)**, they determined the EAAs of soy protein (85.00% protein) and they reported that contained 8.17% leucine, 6.24% lysine, 4.98% valine and 4.90% isoleucine (as a major EAAs), while the lowest values of EAAs (1.28 and 1.35%)

were recorded by cysteine and methionine respectively. Generally, SPI recorded a higher value of BCAAs (18.01 g/100g sample) compared to 8.10 g/100g sample which obtained by SMP. With regard to total AAAs, the highest value (6.61 g/100g sample) was obtained by SPI. Consequently, the Fisher ratio (%) was calculated and the obtained date indicated that, SMP recorded the highest value of Fisher ratio (2.84%) followed by 2.72% for SPI. Accordingly, soy protein has a balance of amino acids, including nine essential amino acids for the human body, similar in composition to milk protein, and is considered to be a protein of high nutritional value **(Ashaolu, 2020)**. Thus, the process of preparing high Fisher ratios from soy protein has been widely reported **(Lu, *et al.*, 2023)**.

Regarding the amino acids content of legume seeds, the lentil seeds recorded 10.26 g/100g sample as total EAAs with lower values of sulfur amino acids (0.17 g/100g sample methionine and 0.03 g/100 sample cysteine), while the highest values of EAAs of lentil were recorded by leucine, lysine, phenylalanine, isoleucine valine and threonine content (2.14, 1.79, 1.53, 1.27, 1.20 and 1.13 g/100g sample, respectively). With regard to the lupine seeds, the highest values of EAAs (2.13, 1.53, 1.41 and 1.30 g/100g sample) were obtained by leucine, valine, isoleucine and phenylalanine, respectively) with 11.22g/100g sample as total essential amino acids. According to the previous study **(Samaranayaka, 2017)**, lentil protein is a good source of essential amino acids leucine, lysine, threonine, and phenylalanine, but has lower content of sulphur-containing essential amino acids methionine and cysteine.

The major EAAs for chickpea was obtained by leucine, isoleucine, phenylalanine and valine, which in a line with **Arab *et al.* (2010)**, they estimated the EAAs content of chickpea flour and they found that leucine, isoleucine, phenylalanine and valine, respectively recorded the highest values of total EAAs. The same results also were obtained by **Zia-Ul-Haq *et al.* (2007)**, they reported that the leucine, isoleucine, phenylalanine and valine are the major content of total EAAs of chickpea flour.

The lowest value (2.24%) of the Fisher ratio was recorded by lupine seeds. Meanwhile, intermediate values of F ratio were obtained by lentil, chickpea (2.45 and 2.64%, respectively). Where, high Fischer ratio oligopeptides (HFROs) are a group of oligopeptides containing high levels of BCAAs and low level of AAAs, where disorders resulting from imbalances in branched-chain amino acids (BCAAs) and aromatic amino acids (AAAs) in the bloodstream, including liver diseases, may be managed through the supplementation of high Fisher ratios via pharmacological agents or dietary sources. Additionally, legumes (soy and lentil) contain proteins and peptides that are thought to be associated with a reduced risk of death from cardiovascular disease **(Mullins and Arjmandi, 2021)**.

Table 4: Amino acids profile and protein quality of used raw materials

AAs (g/100g sample)	Samples				
	Lentil	Lupine	Chickpea	SMP	SPI
Essential amino acids (EAAs)					
Valine	1.20	1.53	1.10	2.01	4.47
Methionine	0.17	0.56	0.34	0.56	1.02
Cysteine	0.03	0.22	0.42	0.25	1.52
Isoleucine	1.27	1.41	1.11	2.04	5.41
Leucine	2.14	2.13	2.11	4.05	8.13
Phenylalanine	1.53	1.30	1.11	1.64	4.50
Lysine	1.79	1.10	1.09	2.43	5.40
Threonine	1.13	1.26	0.77	1.52	3.36
Histidine	0.65	0.75	0.53	1.66	2.02
Tyrosine	0.35	0.96	0.68	1.21	2.11
Non-essential amino acids (NEAAs)					
Aspartic acid	3.20	5.24	1.84	2.46	10.21
Glutamic acid	4.21	6.37	2.69	6.09	13.87
Serine	1.71	4.03	1.05	2.04	4.93
Glycine	1.10	2.30	0.65	0.71	3.56
Arginine	1.89	0.70	1.68	1.11	7.14
Alanine	1.35	1.81	1.01	1.25	4.05
Proline	1.16	2.22	0.80	2.32	4.4
Total EAAs	10.26	11.22	9.26	17.37	37.94
Total NEAAs	14.62	22.67	9.72	15.98	48.16
Total amino acids	24.88	33.88	18.98	33.35	86.09
Total BCAA	4.61	5.07	4.32	8.10	18.01
Total AAAs	1.88	2.27	1.79	2.85	6.61
F (%)	2.45	2.24	2.41	2.84	2.72

Where: SMP= skimmed milk powder, SPI= soy protein isolate, BCAAs= branched chain amino acids, EEAs= essential amino acids, NEAAs= non-essential amino acids, AAAs= aromatic amino acids.

Chemical composition of pasta samples

Pasta samples were estimated for the approximate chemical composition and the obtained data are shown in Table 5 and according to the obtained results it can be seen that the P1 pasta sample (40% soy protein isolate + 10% skimmed milk powder + 30% lentil flour + 10% chickpea flour + 10% lupine flour) recorded 12.50% moisture, 3.90% ash, 53.08% protein, 1.73% fat, 2.29% fiber and 39.01% total carbohydrates. While the P2 pasta sample (40% soy protein isolate, 10% skimmed milk powder, 10% lentil flour, 30% chickpea flour, and 10% lupine flour)

contained 12.37% moisture, 3.90% ash, 51.46% protein, 2.58% fat, 1.89% fiber and 40.19% total carbohydrates. Also, the P3 pasta sample contained 12.56% moisture, 4.26% ash, 54.29% protein, 2.97% fat, 3.31% fiber and 35.16% total carbohydrates.

According to the statistical analysis, no significant differences between all pasta samples concerning the moisture content, while the highest value of ash, protein and crude fiber significantly recorded by the P3 pasta sample. This can be explained by the fact that the sample contains a high percentage of lupins, which recorded the highest percentage of ash, protein and crude fiber (Table 2) compared to other legumes (lentil and chickpea). It is also clear that the P1 pasta sample contains the lowest value of fat content, given that the main ingredient of its formula is lentil flour, which recorded the lowest fat content compared to lupine and chickpeas. Thus, low carbohydrates content of lupine as main components of P3 formula was reflected in total carbohydrate content of P3 pasta sample which recorded the lowest percentage of total carbohydrates compared to P1 and P3 samples.

In general, pasta produced solely from legumes exhibits considerable nutritional benefits, particularly in protein content. However, achieving a 100% legume dough that is suitable for the extrusion process which includes hydration, mixing, and extrusion-poses challenges **(Laleg *et al.*, 2021)**. Numerous researchers suggest that incorporating legume flours into wheat pasta can effectively enhance its nutritional profile **(Osorio-Diaz *et al.*, 2008; Wood, 2009)**. Others (**Teterycz *et al.*, 2020)** explored the effects of adding legume flours on the chemical composition of pasta and their findings indicated that a higher proportion of legume flour significantly enhanced the levels of dietary fiber, ash, protein, and essential amino acids, particularly lysine. According to **Hooper *et al.* (2019)** they reported that dry bean pasta was nutritionally superior to wheat pasta with higher protein, ash, resistant starch and protein digestibility corrected amino acid score as well as lower total starch content.

Table 5: Chemical composition of pasta samples

Components (% on d.w.b.)	Pasta samples			LSD at 0.05
	P1	P2	P3	
Moisture	12.50a±0.45	12.37a±0.42	12.56a±0.17	1.532
Ash	3.90b±0.03	3.90b±0.02	4.26a±0.05	0.155
Protein	53.08b±0.11	51.46c±0.36	54.29a±0.25	1.094
Fat	1.73c±0.06	2.58b±0.03	2.97a±0.04	0.191
Crude fiber	2.29b±0.08	1.89c±0.04	3.31a±0.13	0.370
Total carbohydrates	39.01b±0.07	40.19a±0.28	35.16c±0.04	0.686

All results are expressed as Means ± SD.

Values in each raw which have different letters are significantly different ($p<0.05$).

P1= 40% soy protein isolate + 10% skimmed milk powder + 30% lentil flour + 10% chickpea flour + 10% lupine flour.

P2= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 30% chickpea flour + 10% lupine flour.

P3= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 10% chickpea flour + 30% lupine flour.

Physical properties of pasta

Hardness degree of uncooked pasta

The hardness (N) is related to the strength of structure under compression during the first compression cycle **(Di Monaco *et al.*, 2008)**. Force necessary to attain a given deformation. The hardness of uncooked pasta was determined by a texture profile analyzer and the results were presented in Table

6. The data obtained indicated that, the P1 pasta sample recorded the highest value of hardness (79.89 N) with significant differences compared to other pasta samples; the data also indicated the lowest values of hardness (72.25 and 71.72 N) were observed by the P3 and P2 pasta samples respectively with no significant differences between them. Thus, there is a direct relationship between the hardness of the product and the legume ratios, which might result from the incorporation of protein-rich flour that requires more water to obtain a good dough, leading to an extremely hard texture **(Gularte *et al*., 2012; Huamaní-Perales *et al*.,2024)**. A similar finding by **Gogoi *et al*. (2023)** reported that more strength was needed to break cookies incorporated with legumes flour. Also, **Aziah *et al.* (2012)** reported higher hardness resulted from the incorporation of protein-rich flour which needs more water.

Table 6: Hardness degree of uncooked pasta

Uncooked pasta samples	Texture parameter
	Hardness (N)
P1	79.98a±0.11
P2	71.72b±0.85
P3	72.25b±0.43
LSD at 0.05	2.323

All results are expressed as Means ± SD.

Values which have different letters are significantly different (p<0.05).

P1= 40% soy protein isolate + 10% skimmed milk powder + 30% lentil flour + 10% chickpea flour + 10% lupine flour.

P2= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 30% chickpea flour + 10% lupine flour.

P3= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 10% chickpea flour + 30% lupine flour.

Color attributes of cooked pasta

The color attributes of cooked pasta were measured concerning (lightness, redness and yellowness) L*, a* and b* values respectively. The obtained data are shown in Table 7. According to the obtained results it can be seen that, the highest L* value (more lightness) was recorded by the P3 pasta sample with significant differences compared to P1 (68.48) and P2

(70.14) with no significant differences between them. However, P3 pasta sample recorded the lowest values of redness and yellowness (11.91 and 17.58, respectively) with significant differences. While the highest values of redness and yellowness were recorded by P1 pasta sample (14.50 and 22.26, respectively). **Gallegos-Infante *et al.* (2010)** proved that composite spaghetti pasta is darker in colour after they found that their pasta with more bean flour was darker. Another study by **Martín-Esparza *et al.* (2013)** reported a darker colour was obtained in composite pasta samples due to the legumes flour brown aspect, the colour differences occurring apparently during cooking.

Table 7: Color attributes of cooked pasta

Cooked pasta samples	Color parameters		
	L*	a*	b*
P1	68.48b±0.67	14.50a±0.25	22.26a±0.23
P2	70.14ab±0.08	12.88b±0.11	18.81b±0.21
P3	71.29a±0.56	11.91c±0.13	17.58c±0.37
LSD at 0.05	2.107	0.728	1.168

All results are expressed as Means ± SD.

Values in each column which have different letters are significantly different ($p<0.05$).

P1= 40% soy protein isolate + 10% skimmed milk powder + 30% lentil flour + 10% chickpea flour + 10% lupine flour.

P2= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 30% chickpea flour + 10% lupine flour.

P3= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 10% chickpea flour + 30% lupine flour.

Cooking quality of cooked pasta

Dried pasta is typically consumed after rehydration by cooking to recover its properties. Therefore, it is important to understand the processes occurring during the rehydration of dried pasta, which is a complicated mass transport process governed by several migration mechanisms of water into the pores **(Ogawa and Adachi, 2017)**. Therefore, the effect of different formulas on pasta quality was conducted and the obtained results are shown in Table 8 and from the obtained results it could be observed that, the lowest value of optimum cooking time was investigated by the P1 pasta sample (20.86 min) with significant differences compared to the P2 pasta sample that recorded the highest value of optimum cooking time (24.22 min). With regard to the cooking yield of pasta, the highest values (169.29 and 166.93%) were recorded by P1 and P3 pasta samples respectively with no significant difference between them. Data also indicated no significant differences between all pasta samples concerning cooking loss and nitrogen loss. It is worth noting that, cooking loss is defined as the quantity of solids going into water during the cooking of pasta, which determines the quality of pasta where compact textured pasta leads to lower cooking loss **(Petitot *et al.*, 2010)**. According to **AACC 2000**, all the cooking loss obtained values are within the acceptable limits since the solid loss in cooking water should not exceed 9%. Generally, the extent of protein coagulation and starch gelatinization, and consequently, the overall cooking quality of the final pasta product, is greatly affected by the native properties of protein quantity and quality **(El-Sohaimy *et al.*, 2020)**. In the context of pasta production, gluten is regarded as a critical component influencing cooking quality **(Kaplan, 2022)**.

Table 8: Cooking quality of cooked pasta

Cooked Pasta samples	Quality parameters			
	Optimum cooking time (min)	Cooking yield (%)	Cooking loss (%)	Nitrogen loss (%)
P1	20.86b±0.61	169.29a±1.34	6.69a±0.06	5.17a±0.06
P2	24.22a±0.92	161.93b±1.52	6.82a±0.07	5.28a±0.17
P3	22.16ab±0.25	166.68ab±1.84	6.62a±0.11	5.23a±0.08
LSD at 0.05	2.711	6.572	0.347	0.473

All results are expressed as Means ± SD.

Values in each column which have different letters are significantly different ($p<0.05$).

P1= 40% soy protein isolate + 10% skimmed milk powder + 30% lentil flour + 10% chickpea flour + 10% lupine flour.

P2= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 30% chickpea flour + 10% lupine flour.

P3= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 10% chickpea flour + 30% lupine flour.

Sensory evaluation of pasta

The cooked pasta samples were evaluated by by 18 experienced panalists at the Food Technology Laboratory of the National Research Center, with drinking a quantity of water to separate the samples during tasting to evaluate sensory parameters (color, taste, texture, flavor, appearance and overall acceptability) and the obtained data are shown in Table 9 and according to the statistical analysis the highest score of color was significantly recorded by the P1 pasta sample, while P2 and P3 recorded 16.60 and 16.80 respectively with no significant differences between them. As for the taste and appearance parameters, no significant differences were observed concerning all pasta samples. Generally, P1 pasta sample recorded the highest score of overall acceptability (90.00) with significant differences compared to other pasta samples which recorded lower scores of overall acceptability (85.40 and 85.20) with no significant differences between them (P2 and P3, respectively) such results were agreed with those obtained by **Teterycz *et al.* (2020)** explored the effects of adding legume flours on sensory attributes of pasta and their findings indicated that a higher proportion of legume flour significantly enhanced sensory parameters. Furthermore, the use of a combination of hydrocolloids has been shown to enhance the organoleptic properties of gluten free products. Specifically, the addition of 1% hydrocolloids significantly improved the color, taste, texture, and overall flavor profile of gluten-free products as confirmed by **Thejasri *et al*., (2017)**.

Table 9: Sensory evaluation of pasta

Samples	Sensory parameters					
	Color (20)	Taste (20)	Texture (20)	Flavor (20)	Appearance (20)	Overall acceptability (100)
P1	18.00a±0.67	18.00a±0.52	17.60a±0.84	18.40a±0.67	18.00a±0.67	90.00a±1.49
P2	16.60b±0.52	17.20a±0.94	17.00a±0.72	17.60b±0.52	17.20a±0.94	85.40b±1.71
P3	16.80b±0.79	17.20a±0.67	17.00a±0.79	17.40b±0.50	17.30a±0.79	85.20b±0.79
LSD at 0.05	0.738	0.830	0.808	0.713	0.830	1.536

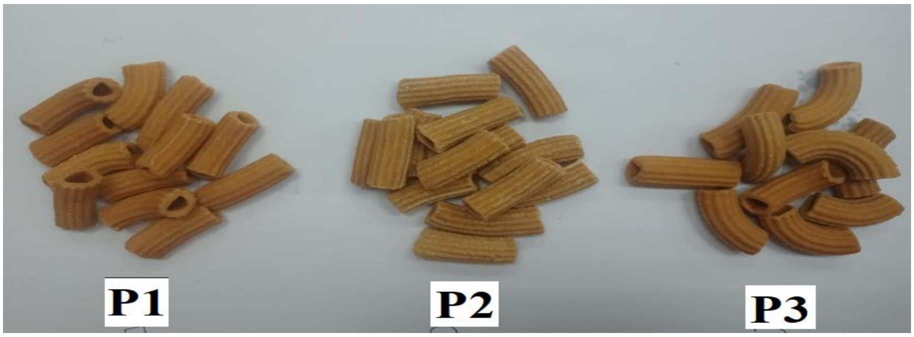
All results are expressed as Means ± SD.

Values in each column which have different letters are significantly different ($p<0.05$).

P1= 40% soy protein isolate + 10% skimmed milk powder + 30% lentil flour + 10% chickpea flour + 10% lupine flour.

P2= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 30% chickpea flour + 10% lupine flour.

P3= 40% soy protein isolate + 10% skimmed milk powder + 10% lentil flour + 10% chickpea flour + 30% lupine flour.



Photographs 1: Uncooked pasta samples

Photographs 2: Cooked pasta samples

Nutritional value of preferred pasta sample

Based on the results of sensory evaluation tests, the most favorable sample was chosen for further specialized analyses focusing on their nutritional value. This included amino acid content, chemical composition, and mineral content. The objective was to ascertain the essential nutrients provided by each meal (200g) that may play a role in the improvement of liver cirrhosis.

Amino acids content and protein quality of preferred pasta sample based on sensory tests

Table 10 illustrated the amino acids profile and protein quality of the preferred pasta sample (P1) based on the organoleptic tests and the obtained data indicated that, it contained leucine (4.72 g/100g sample), lysine (3.50 g/100g sample), isoleucine (3.00 g/100g sample) and valine (2.61 g/100g sample), while the lowest values of EAAs were obtained by methionine (0.60 g/100g sample), cysteine (0.71 g/100g sample), tyrosine (1.23 g/100g sample) and histidine (1.30 g/100g sample).

Table 10: Amino acids profile and protein quality of preferred pasta sample

Amino acids profile of pasta sample (*P1)	
Amino acids	Results (g/100g sample)
Essential amino acids (EAAs)	
Valine	2.61
Methionine	0.60
Cysteine	0.71
Isoleucine	3.00
Leucine	4.72
Phenylalanine	2.37
Lysine	3.50
Threonine	2.04
Histidine	1.30
Tyrosine	1.23
Total EAAs	22.08
Non-essential amino acids (NEAAs)	
Aspartic acid	6.00
Glutamic acid	8.32
Serine	3.20
Glycine	2.12
Arginine	3.77
Alanine	2.43
Proline	2.64
Total NEAAs	28.48

*P1= 40% soy protein isolate + 10% skimmed milk powder + 30% lentil flour + 10% chickpea flour + 10% lupine flour

Concerning the non-essential amino acids (NEAAs) content of the preferred pasta sample it showed the glutamic and aspartic acid as major amino acids respectively, conversely the glycine was found as a limited NEAA. Thus, the incorporation of legume proteins into baked goods has been shown to enhance the protein content and improve the amino acid profile of the final product **(Mohammed *et al.*, 2012)**. A key characteristic of legume proteins is their substantial lysine content, an essential amino acid, alongside a lower concentration of sulphur-containing amino acids. This unique composition positions legume proteins as an excellent complement to traditional cereal proteins, such as those found in wheat, which are typically low in lysine but rich in sulphur amino acids **(Singh *et al.*, 2022)**. **Teterycz *et al.* (2020)** explored the effects of adding legume flours on the chemical composition, of pasta and their findings indicated that a higher proportion of legume flour significantly enhanced the levels of protein, and essential amino acids, particularly lysine.

Protein quality of preferred samples based on sensory tests.

According to the amino acids content of final products, the protein quality was calculated for the preferred pasta sample (P1) and the obtained results were presented in Table 11. Data indicated that it contained 20.67 and 7.19 g/200g pasta samples of BCAAs and AAAs respectively, with 2.87% Fisher ratio. Also, the P1 pasta sample recorded higher values of PERa and PERb (2.47 and 2.51, respectively) with 70.64% biological value.

The protein efficiency ratios (PER) of some legumes are comparable to milk or beef, although different bean species may have different PER values **(Rockland and Radke, 1981; Iqbal, 2022)**. In a study conducted by **Hove *et al.* (1978)** using PER to assess protein quality of selected grain legumes, *Lupinus angustifolius* seeds was found to have growth and PER equal to reference casein and significantly superior to any of the other species (lupin species, *phaseolus* species, peas, and filed beans) when fed raw. However, after cooking, all beans and peas gave very good growth and PER values. Thus, developing countries, especially in the rural areas, can benefit to the application of such mixture of vegetable proteins in the preparation of health foods **(Rama *et al.*, 2018)**. Others **(Holeček, 2017)** showed that BCAA formulas supplemented are among the most useful in an improvement of the nutritional status and associated complications, like liver cirrhosis, mental and physical fatigue, and immune function.

Iwao *et al.* (2020) stated that supplementation of BCAA has a direct effect on fatty liver and liver cirrhosis. Histomorphological findings confirmed that BCAA treatment mitigated fat accumulation in the liver, besides this BCAA treatment decreased an increase in the steatosis score resulting in remarked improvement in liver steatosis. Our findings towards the BCAAs content of preferred sample (P1) are in close to the diet identified by **Tischendorf *et al.* (2019)** as therapeutic meals for patients with cirrhosis of the liver.

Generally, the nutritional management of patients with cirrhosis is focused on ensuring adequate consumption of calories, including proteinaceous foods containing proper quantities of BCAA. Clinical guidelines recommend consumption of 1.2–1.5 g/kg/ day for compensated cirrhotic patients and about 2 g/ kg/day for decompensated cirrhotic patients to counteract the avid protein catabolism **(Warner and Satapathy, 2023).** Other studies showed proper supplementation of BCAA can enhance protein synthesis, attenuate insulin resistance, and improve lipid and glucose metabolism. **(Naseer *et al.*, 2019)** Patients are also advised to supplement vitamins to correct or prevent any vitamin or mineral deficiencies **(Dhaliwal *et al.*, 2021)**.

Table 11: Protein quality per serving (200g pasta sample)

Components	Pasta sample (*P1)	
	Results	Units
Total BCAA	20.67	g/200g sample
Total AAA	7.19	g/200g sample
F ratio	2.87	%
Protein Efficiency Ratio (PER)a	2.47	-
Protein Efficiency Ratio (PER)b	2.51	-
Biological value% (B.V)	70.64	%

*P1= 40% soy protein isolate + 10% skimmed milk powder + 30% lentil flour + 10% chickpea flour + 10% lupine flour

Nutrition quality of preferred pasta sample based on sensory tests

The nutrition quality of preferred pasta sample (based on sensory test) as final product was investigated and the obtained results are shown in Table 12 and according to the obtained results it could be noticed that it contained 106.16g protein, 3.45g fat and 78.01g total carbohydrates per serving (200g pasta sample), with total calories 767.75 kcal per serving. Data also indicated that P1 sample contained 11.37 mg iron, 6.39 mg zinc, 1665.23 mg potassium, 285.03 mg magnesium and 572.15 mg sodium per 200g pasta sample. **Teterycz *et al.* (2020**) explored the effects of adding legume flours on the chemical composition, of pasta and their findings indicated that a higher proportion of legume flour significantly enhanced the levels of dietary fiber, ash and protein content.

Generally, minerals play a crucial role in the metabolism of carbohydrates, fats, and proteins, in addition to being integral to the processes of DNA and RNA replication. They function as antioxidants, contribute to the development of healthy bone structure, support immune system health, promote good vision, facilitate normal fetal growth, and are vital for neuromuscular activity **(Saltman and Strause, 1993)**. Furthermore, these minerals act as activators for numerous enzymes that are essential for sustaining life **(Uddin *et al.*, 2016**). Others **(Sun *et al.*, 2014; Mohammad *et al.*, 2012*)**, methioned that, some minerals such as zinc and iron improves liver function and their deficiency can lead to a detrimental effect on liver function. As reported by **Giuberti *et al.* (2015)**, legumes have been recognized as valuable components in the formulation of snacks and baked goods, such as biscuits, bread, and pasta, particularly in the context of gluten-free product development **(Giuberti *et al.*, 2016).** The addition of legume flour significantly enhances the nutritional profile by increasing levels of protein, dietary fiber, resistant starch, and the predicted glycemic index.

Thus, **Lai *et al.* (2022)** stated that, sodium restriction may reduce the palatability of food, representing a barrier to adequate nutrition intake. In a study of 120 outpatients with cirrhosis and ascites, only 31% were adherent to a 2-g-sodium diet, and adherent patients had a 20% lower daily caloric intake. When patients are prescribed a sodium-restricted diet, it should be balanced with educational resources that offer suggestions to improve diet palatability. Liberalization of sodium restriction should be considered if the patient is unable to maintain nutritional targets because of diet unpalatability.

Table 12: Nutrition quality per serving (200g sample)

Components	Pasta sample (*P1)	
	Results	Units
Total protein	106.16	g/200g
Total carbohydrates	78.01	g/200g
Total fat	3.45	g/200g
Calories	767.75	kcal/200g
Iron	11.37	mg/200g
Zinc	6.39	mg/200g
Potassium	1665.23	mg/200g
Magnesium	285.03	mg/200g
Sodium	572.15	mg/200g

*P1= 40% soy protein isolate + 10% skimmed milk powder + 30% lentil flour + 10% chickpea flour + 10% lupine flour

Conclusion

The increasing popularity of this trend presents the pulse industry with a chance to explore innovative applications for whole pulses as functional foods where the prepared legumes pasta was discribed as higher protein, BCAAs and other minerals compared to traditional pasta, which in turn helps reduce some diseases, especially liver diseases. Additionally, it allows for the creation of ingredients and products that can be integrated with various legumes, facilitating the development of convenient alternative food items and industrial products.

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(*) الأسماء مرتبة ترتيباً ابجديًا.