Methods of Protein Extraction from House Crickets (Acheta domesticus) for Food Purposes

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Article

Methods of Protein Extraction from House Crickets (Acheta domesticus) for Food Purposes

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Abstract: Global population projected to reach 10 billion by 2050 emphasizing the need for sustainable food production. Traditional protein sources present environmental and scalability challenges, demanding the diversification of protein sources. Edible insects, such as house crickets (*Acheta domesticus*), emerged as a promising solution due to their high nutritional value, efficient feed conversion rates, and lower environmental impact compared to conventional protein sources. Incorporating insect powders into new food products can improve consumer acceptance, but often lead to poor functionality and/or undesirable organoleptic characteristics. However, protein isolates were revealed to be effective in enhancing functionality and consumer acceptance. This study aimed to develop and compare the yield of three different protein extraction methods, using sodium hydroxide, ascorbic acid or alcalase, from house crickets (*Acheta domesticus*) for food applications. Protein extraction was performed on cricket powder with a mean protein content of 57.95 g/100 g, and the results were evaluated. The enzymatic method showed the highest protein extraction rate of 85.97% with a mean protein content of 74.03 g/100 g, while extraction with NaOH or ascorbic acid resulted in rates of 74.32 and 56.99%, respectively. Further studies on the functionality and sensorial evaluation of products developed with this protein extract are recommended.

Keywords: edible insects; crickets; protein extraction; sustainability

1. Introduction

In recent years, global hunger levels have reached an alarming scale, with projections indicating a growing trend affecting more and more people around the world. This urgency demands immediate and serious action from all nations. According to the Global Report on Food Crises (GRFC) in 2022 [1], the situation has escalated to unprecedented levels: approximately 193 million people in 53 countries/territories suffered from severe food insecurity in 2021, requiring urgent assistance. This represents an alarming increase of nearly 40 million people compared to 2020 [2], and a staggering 22% growth in acute food insecurity from 2020 to 2021 due to population increase. Since 2016, there has been an 80% increase in the number of people experiencing severe food crisis conditions, as revealed by the six editions of the GRFC. The Food and Agriculture Organization (FAO) projects the global population will reach 9.1 billion by 2050, which will require twice the current food production to feed the world, necessitating innovative approaches to ensure adequate food production [3,4].

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Traditional protein sources like soybeans, fish, and meat face environmental and scalability challenges [5]. FAO reports from 2021 [6], critically emphasize the impact of traditional practices, including biodiversity degradation, ecosystem damage, and significant contributions to climate change through carbon emissions. Boccardo et al. (2023) highlights that animal protein production, a major component of this demand, is known to have the highest environmental impact in current food production systems [7]. This highlights the need to question and reassess the sustainability of current agrifood systems. Moreover, global dietary trends favoring high consumption of sugar, fat, and meat correlate with increasing rates of chronic non-communicable diseases like obesity and diabetes. These dietary patterns adversely affect public health and contribute to rising healthcare costs worldwide, reflecting a misalignment between current food consumption patterns and optimal health outcomes [8].

Among the three major macronutrients, the predicted demand for proteins has sparked a range of concerns, most notably whether its supply can be met by harvesting from traditional sources of protein alone, such as livestock [9]. Given the challenges referred above, it becomes clear that innovative approaches are required to sustainably meet the growing protein demands of the human population. The alternative protein industry is poised to play a significant role in addressing this demand, and through ongoing research, innovation, and investment, it has the potential to contribute to a more sustainable and resilient food system [10].

In this context, edible insects, particularly crickets, have emerged as a promising solution to these challenges due to their rich nutritional profile, efficient feed conversion rates, and lower environmental footprints compared to some traditional protein sources [11,12]. A further advantage of insects as a food source is the high percentage of the animal that can be consumed; up to 80% of a cricket is edible for humans, compared to 55% for pigs and chickens and 40% for cattle [13]. A case study report by the European Union (EU) estimated that by the year 2054, alternative proteins will make ≤ 33% of the global protein consumption, of which insects will account for ~11% [14].

House crickets (*Acheta domesticus*) are expected to play an important role in the future food systems presenting unique opportunities for improving food and nutritional insecurity status of both resource-poor and Western populations [15,16]. House crickets are particularly notable for their high protein content (with a higher bioavailability) which ranges from 48.06 to 76.19 g/100 g on a dry basis, along with favorable amino acid profiles [12,17]. This makes them an excellent source of protein, comparable and often superior to traditional animal protein sources such as chicken and beef. The protein in house crickets includes essential amino acids (apart from the possible exception of methionine/cysteine) necessary for various bodily functions, including muscle repair and enzyme production [18,19]. Proteins from crickets include albumin, globulin, glutelin, and prolamin, with albumin and globulin being the most prominent [19]. These proteins can significantly contribute to the dietary protein needs of different age groups, potentially covering 100% of the daily recommended intake for essential amino acids, in both children and adults [12].

Regarding environmental benefits of producing crickets for food, various studies have shown the fewer resources are needed compared to traditional protein sources, such as water, feed and space, to produce the same amount of biomass [20, 21].

Insects in general have short life cycles, and this makes them highly efficient. For instance, crickets are excellent bio converters which can be fed on low value organic by-products of the food industry and transform it into high quality food [20]. In addition, the by-products of insect production, including frass (insects' excrement and exoskeletons), are high-quality crop amendments which could reduce the need to produce and apply nitrogen fertilizers [22].

Despite their benefits, the widespread acceptance of edible insects in Western diets remains a challenge due to cultural and psychological barriers and consumer acceptance of insects as a direct food source remains low in Europe [23-25]. Studies have consistently shown that Western consumers often respond with feelings of disgust and neophobia when presented with the idea of consuming whole insects. This cultural barrier has led to a strategic shift in the industry towards incorporating insects in more familiar food forms [20]. Enhanced technological functionality in developing insect-based ingredients plays a significant role in providing familiar food products that appeal to

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consumers [26]. Research indicates that there is a higher acceptance of insect-based ingredients when they are not visible, such as in the form of powders used in various food products like protein bars, baked goods, and pasta [24]. This has resulted in the development of insect powders, particularly those derived from *Acheta domesticus* crickets, due to their potential as a food ingredient and approval for human consumption by the European Commission [27]. Although insect powders can be used to formulate new products, several challenges have been encountered in the final products. Despite powders help in overcoming the visual and psychological barriers associated with whole insect consumption, they come with their own set of challenges. Studies have highlighted several issues related to the functional properties of insect powders. For instance, insect powders have been reported to have poor solubility and emulsification properties, which can limit their use in food product formulations [24]. Pilco-Romero et al. [12], found that organoleptic characteristics of these powders are sometimes undesirable, affecting the overall appeal of the final food products.

Pan et al. [28] refers that one way to address these issues is through the fractionation of components in insect powders, such as protein isolation. This process can significantly enhance the solubility, emulsification, and foaming properties of insect proteins, making them more suitable for incorporation into various food products [12].

There are several methods to extract protein from insects, including conventional (e.g., solvents, alkali) and advanced or green extraction methods (e.g., enzyme-assisted extraction) [28]. Protein concentration and isolation from insect powders include also different processes, such as defatting, protein solubilization, and isoelectric precipitation. However, the methodologies and conditions selected for each step varied considerably depending on the insect [29]. Research on extracting protein methods has also encountered issues, mainly determining the best method for the insect species to obtain higher yields of protein extraction. Indeed, one significant challenge is the variability in protein yield from different extraction methods. This yield can vary significantly depending on the specific method used, the processing conditions, and the insect species [28].

This study aims to develop and select a method to extract protein content from crickets *Acheta domesticus* for food applications by comparing three different methods, based on their yields of protein extraction and suitability for industrial-scale use.

2. Materials and Methods

2.1. Chemicals

Ethanol, sodium hydroxide (NaOH), ascorbic acid, hydrochloric acid (HCl) and alcalase (Protease from *Bacillus licheniformis*, ≥2.4 U/g) were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Crickets

2.2.1. Crickets rearing and harvesting

Whole, frozen, and unpasteurized crickets (*Acheta domesticus*) were obtained from EASYPROTEIN, located in Santarém, Portugal. Crickets were reared in under controlled temperature (approximately 30 ^{0}C), humidity (50-55 %). To ensure biosecurity and prevent external contamination, all personnel wear personal protective equipment. Crickets were kept in plastic containers measuring 55x39x42 cm with population density carefully managed; to promote well-being egg carton trays were placed inside the boxes [30]. The diet consisted of a commercial poultry feed containing 20.2% of crude protein provided *ad libitum*.

Harvesting occurred at the end of the cricket's life cycle (adult stage), with an average of 59 days. Post-harvest, the crickets were sieved and subsequently euthanized by freezing (-18^{0}C), a process lasting a minimum of 24 hours, and remained there until further processing.

2.2.2. Processing cricket into powder

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The crickets were processed following established protocols with minor adjustments for optimization. The frozen crickets were defrosted, cleaned to remove extraneous materials, and ground them into a paste. This paste was then subjected to drying and further grinding to produce a fine, uniform powder, which was stored under vacuum for further analysis.

2.3. Study design

Three distinct protein extraction methods were evaluated and compared for their efficiency and effectiveness. The first method involved protein extraction using NaOH, following a modified protocol by Zhao et al. [31]. The second method adopted a protein extraction technique using ascorbic acid described by Amarender et al. [32]. The third method employed an enzymatic approach to protein extraction using alcalase (Protease from *Bacillus licheniformis*), based on the methodology outlined by Hall et al. [33]. Each extraction method was performed in triplicate to ensure the reliability of the results. For each method, the composition of lipid, carbohydrates, fiber, ash, and moisture content was also assessed (Table 1).

Table 1. Experimental design for protein extraction methods.

Method of extraction		Group	Proximate analysis (samples number)					
			Protein	Lipids	Carbohydrates	Fiber	Moisture	Ash
1	Alkaline	Experimental	3	3	3	3	3	3
		Control*	5	5	5	5	5	5
2	Acidic	Experimental	3	3	3	3	3	3
3	Enzymatic	Experimental	3	3	3	3	3	3

* The control was the raw sample without extraction (common to the 3 methods, always a total of 5 samples).

2.4. Protein extraction

Prior to the protein extraction from cricket powder, a lipid extraction process was implemented to enhance protein yield, as described by Gravel & Doyen [34]. This lipid removal step was adapted from the method outlined by Quinteros et al. [35], incorporating specific modifications to optimize efficiency.

The defatting procedure consisted in mixing cricket powder with ethanol in a 1:10 weight/ volume (w/v) ratio, under constant magnetic stirring for 1 h at room temperature, to ensure thorough lipid solubilization. Following this, the ethanol-lipid mixture was separated from the cricket powder, via vacuum filtration. The filtered cricket powder was then dried to achieve complete drying and removal of residual ethanol. The defatted cricket powder was then used for protein extraction.

2.4.1. Method 1: Protein extraction with NaOH

A NaOH solution (0.5 M) was mixed with the defatted cricket powder in 50 mL centrifuge tubes at a 6:1 solvent-to-powder ratio (v/w). The tubes were vortexed and placed in a water bath at 40 ^{0}C for 60 min and vortexed every 15 min during the extraction process.

The tubes were then centrifuged at 4 ^{0}C for 20 min at 3500 g, and supernatant and any gel layer formed were removed and preserved for subsequent protein recovery steps. A second extraction was performed on the remaining solid insoluble pellet by repeating the vortex and centrifugation steps. The supernatant and gel layer from the second extraction were also saved for protein recovery. The solid pellet was then kept at - 80 ^{0}C for posterior freeze-drying, to reduce the moisture content to less than 5 % (w/w).

For the recovery of protein potentially lost in the supernatant and gel layer, the collected liquids were adjusted to a pH of 4.3~4.5 using a HCl solution (2 M), which was followed by centrifugation at 4 ^{0}C for 15 min at 2 500 g. The resulting protein precipitate was washed with distilled water and subjected to another centrifugation under the same conditions. In the alkaline medium, proteins are more soluble, hence the adjustment of the pH allowed for precipitation and recovery of these

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potentially lost proteins. The obtained pellet was frozen at – 20 ^{0}C until it could be transported for freeze-drying.

Final extracts were mixed for further analysis and yield calculations.

2.4.2. Method 2: Protein extraction with ascorbic acid

A solution of ascorbic acid (0.5 M) was added to the defatted cricket powder in 50 mL centrifuge tubes at a 6:1 solvent-to-powder ratio (v/w).

The tubes were vortexed before being placed in a water bath of 40 ^{0}C for 60 min and vortexed every 15 min to ensure maximum interaction between the defatted cricket powder and the ascorbic acid.

After this step, the tubes were centrifuged at 4 ^{0}C for 20 min at 3500 g and the supernatant and any resultant gel layer were discarded.

An obtained solid insoluble pellet was subjected to a second extraction, which involved a new addition of the ascorbic acid solution (in the same ratio of the first extraction), vortexing and repeating the centrifugation steps, with the supernatant and gel layer formed during this second extraction also being discarded.

The final insoluble pellet was subsequently frozen at -80 ^{0}C, prepared for freeze-drying to achieve a moisture content of less than 5% (w/w).

Final extracts were mixed for further analysis and yield calculations.

2.4.3. Method 3: protein extraction with enzyme

Alcalase (protease from Bacillus licheniformis) was used for enzymatic protein extraction from defatted cricket powder. The pH was adjusted to the optimal range for alcalase activity, and the samples were subjected to controlled enzymatic hydrolysis under specified conditions. After the hydrolysis, the enzyme was inactivated by heating.

The mixture was centrifuged, and the supernatant was collected, frozen, and freeze-dried. A second centrifugation was performed on the remaining solid pellet, and the resulting supernatants were also freeze-dried. The freeze-dried samples were pooled for further analysis.

Extraction yield and rate were calculated based on the data obtained from proximate composition.

2.4.4. Extraction yield and rates

The extraction yield is the proportion of material retained after the lipid extraction process, relative to the original amount of material. The extraction yield was calculated using equation 1.

$$\text{Extraction yield (\%)} = [(\text{Defatted powder (w)}/\text{Initial powder (w)}]\text{x}100 \quad (1)$$

Where:

Defatted powder refers to the mass of cricket powder remaining after lipid extraction.

Initial powder is the original mass of cricket powder before any extraction.

Protein extraction rate (%) measures the efficiency of the protein extraction process. It considers the amount of protein obtained from the defatted cricket powder, relative to the protein content of the initial material, adjusted by the yield of the extraction process, according to equation 2.

$$\text{Protein extraction rate (\%)} = [\text{Protein content in extract (\%)}/\text{Protein content in defatted powder (\%)}] \text{ x Extraction yield (\%)} \quad (2)$$

Where:

Protein content in extract is the percentage of protein in the extract obtained from the defatted cricket powder.

Protein content in defatted powder is the percentage of protein in the defatted powder before the protein extraction process.

2.5. Nutrient composition

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Proximate composition was conducted on cricket powder without any extraction, as well as on the freeze-dried protein extracts obtained from each protein extraction method, for comparation purposes. Nutritional components were analyzed according to Association of Official Analytical Collaboration (AOAC) [36]. This analysis included, the crude protein content (950.36) determined using the Kjeldahl method, with a protein-to-nitrogen conversion factor of 6.25; fat content (935.38) was measured through Semi-Automatic Soxhlet extraction.; Carbohydrates were calculated by difference (100 - [Protein (%) + Fat (%) + Moisture (%) + Ash (%) + Fiber (%)]) and fiber (950.37) was analyzed using Weende Method. Finally, moisture content was assessed through gravimetric analysis (935.36) and ash content determined by incinerating the samples to dry ash, also followed by gravimetric measurement (930.23).

2.6. Statistical analysis

The results were statistically evaluated using Statistica version 7.0 (StatSoft Inc.). An analysis of variance (ANOVA) with one factor (sample) was performed. The Wilks' significance test was applied to check for homogeneity, at a significance level of 5%. For each dependent variable (protein, lipids, carbohydrates, fiber, ash, and moisture), the mean and standard deviation (LS Mean) were calculated for each condition and the Fisher LSD *post hoc* test was applied to compare means of homogeneous groups.

3. Results

3.1. Nutrient composition

3.1.1. Cricket powder (control)

Among the analyzed nutritional components, protein content (57%) was the highest, followed by fat (19%), fiber (9%), carbohydrates and moisture (6%), and finally ash (4%) (Table 2).

Table 2. Distribution of the nutritional components analysis on the cricket powder (control sample). Results are expressed as % w/w (mean ± sd) (N=5).

Nutritional components of cricket powder (%w/w), dry basis	
Protein	57±0.23
Fat	19±0.1
Carbohydrates	6±0.95
Fiber	9±0.57
Moisture	6±0.03
Ash	4±0.09

3.1.2. Cricket protein extracts

Related to protein content in the extracts, the enzymatic method (sample 3) showed the highest value which was (74.03 ± 0.95) g/100 g. The alkaline hydrolise method used for sample 1 yielded better results than the one used for sample 2 (ascorbic acid), with average crude protein contents of (66.33 ± 1.29) g/100 g and (55.25 ± 0.21) g/100 g, respectively; the protein content in sample 2 (ascorbic acid) was lower than that of the initial control sample. The differences among all samples were significant ($p<0.05$) (Figure 1).

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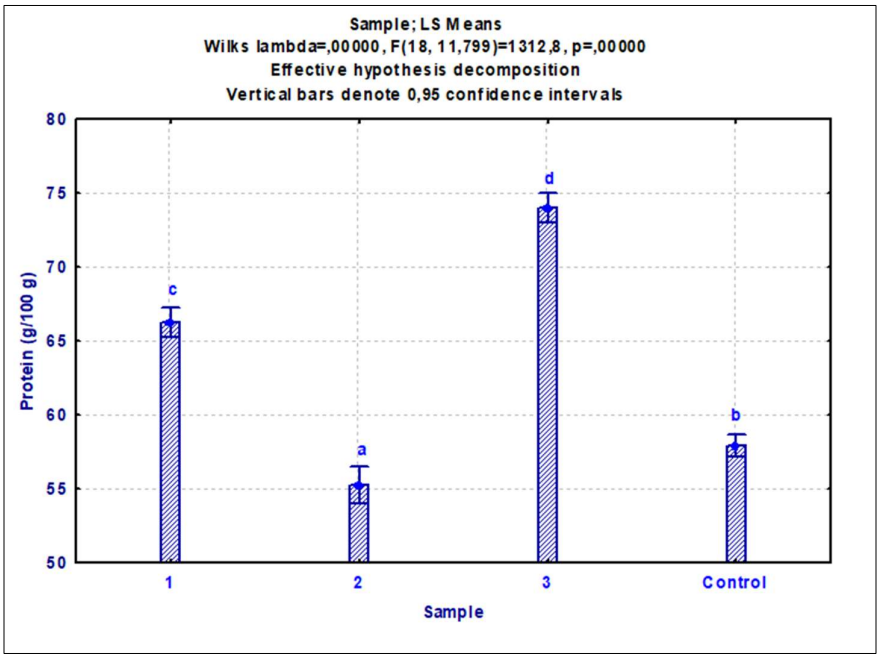

Figure 1. Protein, as g/100 g of cricket powder, in control and after the three extraction methods; sample 1 refers to the chemical extraction with NaOH, sample 2 refers to the chemical extraction with ascorbic acid and sample 3 refers to the enzymatic extraction with alcalase. Data are expressed as (mean ± sd). a, b, c or d is significantly different ($p<0.05$) from control, samples 1, 2 or 3, respectively.

Protein extraction for all methods (1, 2 or 3) resulted in a significant ($p<0.05$) lower lipid content than control (19.51 ± 0.1), with values of (5.26 ± 0.2) g/100 g, (0.56 ± 0.03) g/100 g, and (1.66 ± 0.3) g/100 g, respectively (figure 2).

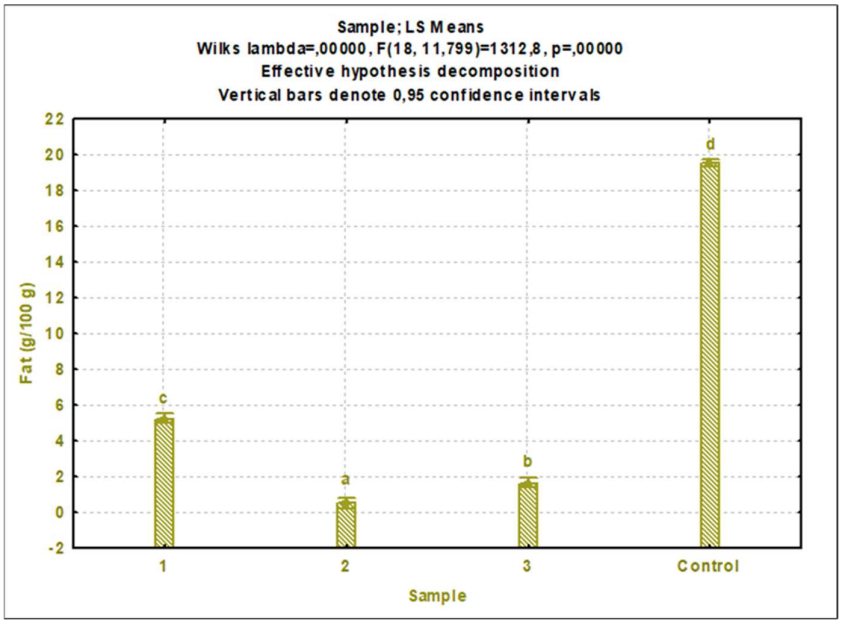

Figure 2. Fat, as g/100 g of cricket powder, in control and after the three extraction methods; sample 1 refers to the chemical extraction with NaOH, sample 2 refers to the chemical extraction with ascorbic acid and sample 3 refers to the enzymatic extraction with alcalase. Data are expressed as (mean ±sd): a, b, c or d is significantly different ($p<0.05$) from control, samples 1, 2 or 3, respectively.

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For carbohydrate content significant ($p<0.05$) differences were observed among all samples and in the following decreasing order (26.37 ± 1.39) g/100 g (sample 2), (10.78 ± 0.33) g/100 g (sample 3), (6.75 ± 0.95) g/100 g (control), and undetectable (ND) values (sample 1) (Figure 3).

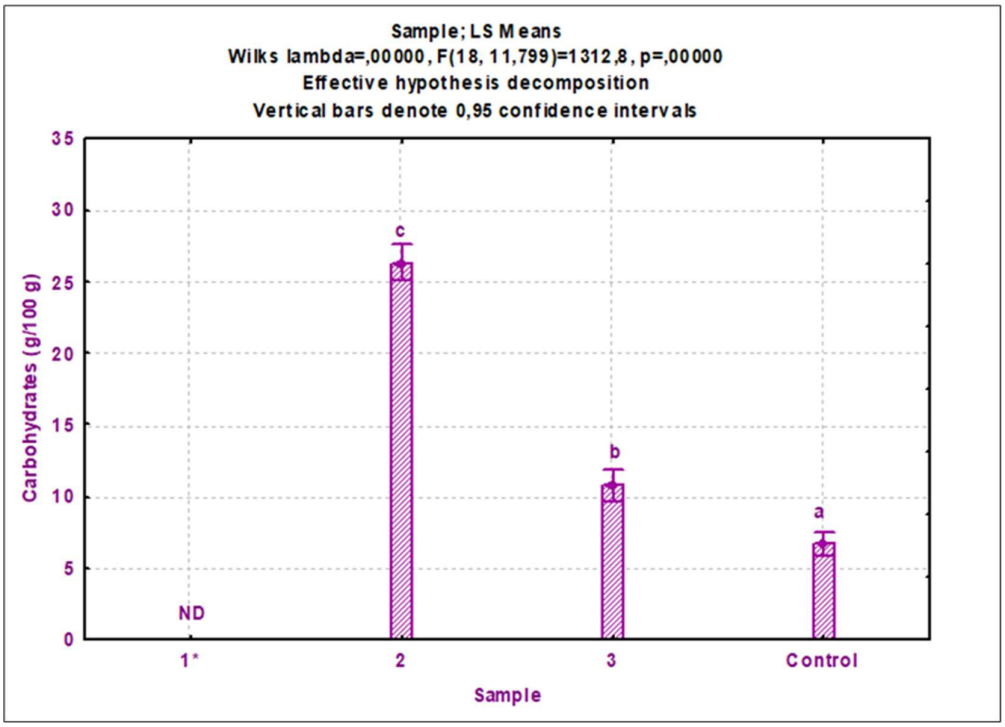

Figure 3. Carbohydrates, as g/100 g of cricket powder, in control and after the three extraction methods; sample 1 refers to the chemical extraction with NaOH, sample 2 refers to the chemical extraction with ascorbic acid and sample 3 refers to the enzymatic extraction with alcalase. Data are expressed as (mean ± sd). a, b, c or d is significantly different ($p<0.05$) from control, samples 1, 2 or 3, respectively. ND – not detectable.

Fiber content in sample 1, obtained from the alkaline hydrolysis, was the highest (20.32 ± 1.46) g/100 g of all the samples ($p<0.05$), followed by sample 2 (11.51 ± 1.19), extracted with ascorbic acid. In contrast, sample 3, resulting from the enzymatic extraction with alcalase, had the lowest fiber content, (0.70 ± 0.45) g/100 g, which was significantly ($p<0.05$) lower than the control (9.51 ± 0.57) g/100 g (Figure 4).

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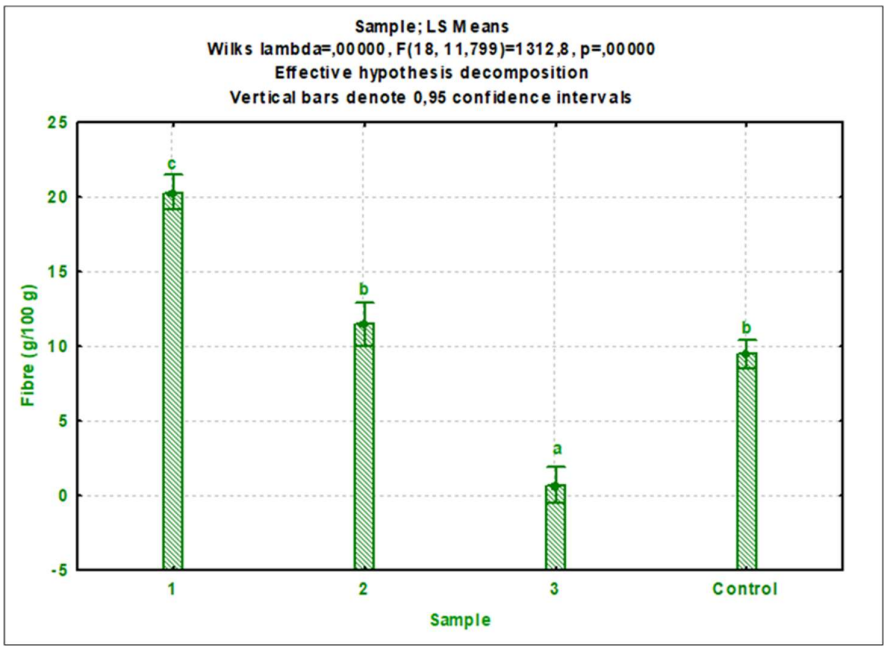

Figure 4. Fiber, as g/100 g of cricket powder, in control and after the three extraction methods; sample 1 refers to the chemical extraction with NaOH, sample 2 refers to the chemical extraction with ascorbic acid and sample 3 refers to the enzymatic extraction with alcalase. Data are expressed as (mean ± sd). a, b, c or d is significantly different ($p<0.05$) from control, samples 1, 2 or 3, respectively.

Regarding ash content, both samples 1 and 3 (extractions with NaOH and with alcalase) showed significantly ($p<0.05$) higher values than control, respectively (9.78 ± 0.77) g/100 g, (8.93 ± 0.78) g/100 g and (4.43 ± 0.03) g/100 g. Sample 2 (extraction with ascorbic acid) had the lowest ash content of all the samples ($p<0.05$), in the protein extract, (1.70 ± 0.03) g/100 g (Figure 5).

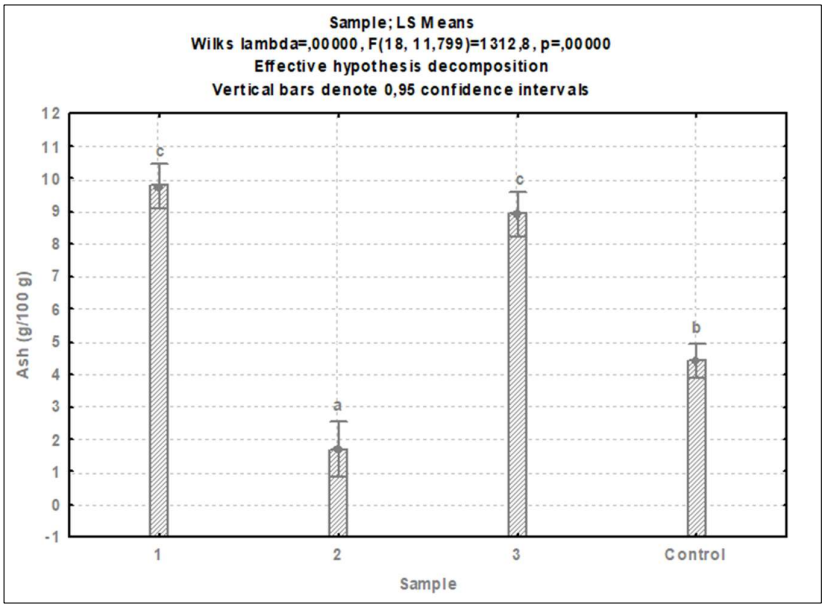

Figure 5. Ash, as g/100 g of cricket powder, in control and after the three extraction methods; sample 1 refers to the chemical extraction with NaOH, sample 2 refers to the chemical extraction with ascorbic acid and sample 3 refers to the enzymatic extraction with alcalase. Data are expressed as (mean ± sd). a, b, c or d is significantly different ($p<0.05$) from control, samples 1, 2 or 3, respectively.

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All moisture content values were statistically different from each other ($p<0.05$). The average values obtained for samples 1, 2, and 3 were (1.26 ± 0.26) g/100 g, (4.55 ± 0.07) g/100 g and (3.9 ± 0.17) g/100 g, respectively and lower than the value found in control (6.05 ± 0.09) g/100 g (Figure 6).

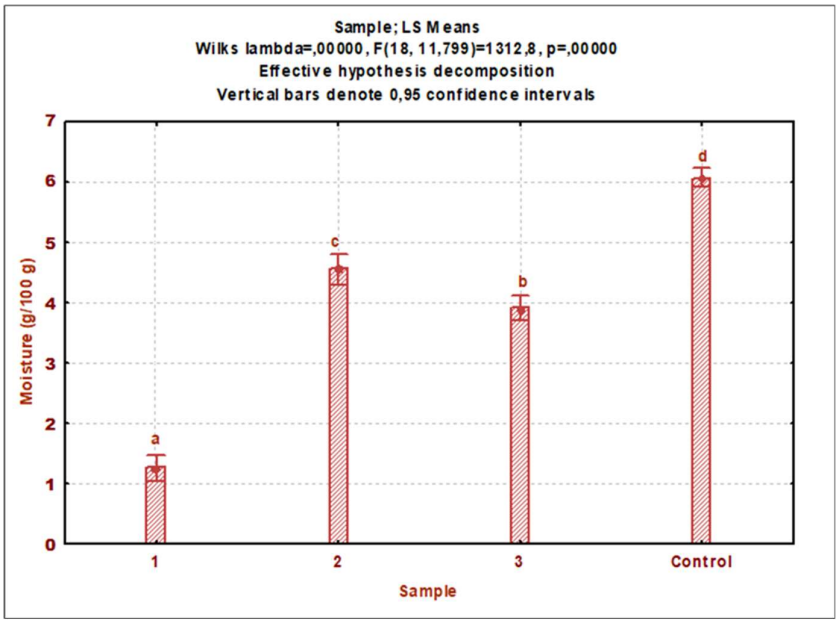

Figure 5. Moisture, as g/100 g of cricket powder, in control and after the three extraction methods; sample 1 refers to the chemical extraction with NaOH, sample 2 refers to the chemical extraction with ascorbic acid and sample 3 refers to the enzymatic extraction with alcalase. Data are expressed as (mean ± sd). a, b, c or d is significantly different ($p<0.05$) from control, samples 1, 2 or 3, respectively.

3.2. Yields and extraction rate

The extraction yields calculated according to equation 1, varied between 69.10-77.81 %, with sample 2 having the lowest value and sample 3, the highest. The protein extraction rates (equation 2) varied between 56.99 and 85.97 %, with sample 2 having the lowest result as it also had the lowest protein content in the final extract. Sample 3 had the highest protein extraction rate compared to samples 1 and 2. Among the samples obtained, sample 3 showed the best results in yield and protein content of the final extract (Table 3).

Table 3. Extraction yield (mass lost after lipid extraction from the initial mass), protein content and protein extraction rate of protein, from defatted cricket powder.

Method	Extraction Yield (%)	Protein (g/100 g)	Protein extraction rate (%)
1 NaOH	75.07	66.33	74.32
2 Ascorbic acid	69.10	55.25	56.99
3 Alcalase	77.81	74.03	85.97

4. Discussion

4.1. Nutritional composition of cricket powder

The crude protein content of the cricket powder, obtained in this research, had an average value of (57.95 ± 0.23) g/100 g (Figure 1), which is lower than the (65.8 ± 0.52) g/100 g reported by Bassett et al. [37]. This lower value can be justified by the difference in harvesting ages, as the crickets used in this study were harvested with 59 days (around 8 weeks), whereas those used by Bassett et al. [37] were about 5-6 weeks old. However, Ndiritu et al. [38] reports that crickets harvested with 10 weeks old, fed with a diet containing 14-21 % protein and supplemented with fresh vegetables (green leaves), achieved a similar protein content of (59.84 ± 1.64) %, after processing into powder (freeze-

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drying and grinding). Besides age, the diet and the nutrients available to the insects throughout their lifecycle can significantly influence their growth rate and nutritional composition. Moreover, under optimal environmental conditions, it is possible to optimize the diet and harvesting age of insects to obtain crickets with higher nutritional value [39, 40].

The average fat content was (19.51 ± 0.1) g/100 g (Figure 2), similarly to Bassett et al. and Ndiritu et al. [37,38], respectively (18.1±0.15) % and (18±0.07) %, in a dry basis. The lipid content in *Acheta domesticus* crickets can vary between 8.9 to 43.9%, in a dry matter basis [12]. The other components, such as carbohydrates, fiber, and ash, were (6.75 ± 0.95) g/100 g, (9.51 ± 0.57) g/100 g, and (4.43 ± 0.03) g/100 g, respectively (Figures 3, 4 and 5) and similar values were found for carbohydrates and ash content by Ndiritu et al. [38], respectively (6.39±1.67) %, (4±0.06) %; a lower fiber content of (7.16±1.26) %, was found by the same authors. The carbohydrate content in *Acheta domesticus* crickets is known to be low, varying between 1.6 to 10.2 g/100 g, similar with the value obtained in the cricket powder in this study. The ash content found in cricket powder agrees with the value reported by Pilco-Romero et al. [12], varying between (1.10-5.60) g/100 g dry basis, showing the potential of *Acheta domesticus* crickets to serve as a source of minerals. In addition, *Acheta domesticus* can also be a source of dietary fiber whose content varies between 3.9 and 7.5 g/100 g (dry basis) [12]. Although in this study it was found a higher value for fiber content, this could be due to the age of the harvested crickets, because it may vary depending on development stage [41]. It is likely that when crickets achieve the adult stage, they do not need to change their exoskeleton (molt) and hardness. The fiber in insects is present in the exoskeleton, where its main component is chitin. It is of notice that studies have demonstrated benefits in the human diet resulting from the presence of chitin in insect- derived powders, such as acting as a prebiotic fiber [12, 42]. Moreover, insect chitin can be converted into chitosan. According to Ayensu et al. [43], chitosan exhibits high bioactivity and interesting properties such as antimicrobial and antioxidant effects.

4.2. Nutritional composition of cricket protein extracts

4.2.1. Protein

One way to improve the acceptance and functionality of insect powders is through the fractionation of its different components into extracts, such as protein isolation. The use of the enzymatic method led to the highest protein content, (74.03 ± 0.95) g/100 g, which is slightly higher than the 70.6 ± 0.01 found by Hall et al. [33]. Such indicates that the modifications made to the method described by Hall et al. (2017) during this work were effective in optimizing the parameters to control during extraction. Even so, the method used by Hall et al. [33] was carried out with a different cricket species (*Gryllodes sigillatus*), and the species can be a variation factor in the protein content [19]. Other factors, such as differences in diet or harvesting age, 6 weeks and 8 weeks, respectively, in Hall et al. [33] and in the present study, may have influenced the results obtained. Trinh & Supawong [44] obtained higher protein content in the extract using the same method as Hall et al. [33] and under the same conditions of (85.9 ± 0.7) % (w/w). It is possible that in the latest study, the base material (crickets) had a higher protein content, providing more protein material for the enzyme to act on, during hydrolysis. Protein content in *Acheta domesticus* crickets can vary between 48.06 and 76.19 g/100 g according to the existing literature [12]. This shows that the higher the initial protein content in the raw material, the higher the protein extraction yield (above 90%), which means that the extraction method is feasible for industrial scale application.

According to Figure 1, the protein extraction with NaOH provided better results than the use of ascorbic acid, where the average crude protein content was (66.33 ± 1.29) g/100 g and 55.25 ± 0.21 g/100 g, respectively. The protein content of the extracts with NaOH is lower than that of Zhao et al. [31], who obtained 79.0%, on a dry basis. This may be due to the lower concentration of NaOH used in this work and the NaOH solution-to- powder ratio of 0.5 M NaOH and 6:1 (v/w). Even so, there was an increase in protein content compared to the control sample from 57.95 to 66.33 g/100 g dry basis.

 doi:10.20944/preprints202411.0190.v1

Regarding the extraction with ascorbic acid, a lower value was obtained compared to Amarender et al. [32], who reported 69.69% dry basis. The protein content was also lower than that of the initial material (control sample), suggesting that meaningful protein losses occurred during the extraction process, possibly in the supernatant that was discarded. In contrast, the protein lost in the supernatant was recovered when using NaOH, leading to a higher protein content in the final extract. It was expected that the use of ascorbic acid would cause protein precipitation in an acidic medium [45], with the proteins remaining in the solid, insoluble part of the pellet rather than in the supernatant after centrifugation. In this study, protein concentration in the pellet was not observed. It should also be noted that protein precipitation in an alkaline medium resulted in a product with higher protein content compared to the acidic method.

4.2.2. Other nutritional components

It was expected that the cricket protein extracts, regardless of the extraction method used, would have a lower lipid content compared to the initial mass (control sample). In accordance, the results showed a significant reduction ($p<0.05$) in lipid content in all methods, with values of 5.26, 0.56, and 1.66 (g/100 g) for fat content, respectively (figure 2). The results for chemical extraction were much lower than those found by Amarender et al. [32] for fat content, which were 13.34 and 7.62% (w/w), respectively. As for enzymatic extraction, the lipid content was lower compared to the (4.8 ± 0.1) % on a dry basis found by Hall et al. [33].

The results demonstrate the effectiveness of the lipid extraction method prior to protein extraction which involves removing lipids from cricket powder.

For carbohydrate content upon all extraction methods and control, significant differences were observed between them ($p<0.05$). Extraction with NaOH resulted in undetectable (ND) values. This may be because NaOH breaks the bonds between proteins and carbohydrates [46, 47]; during centrifugation, the carbohydrates were possibly eliminated in the supernatant. Figure 3 shows that extraction with ascorbic acid leads to the highest carbohydrate values, including control, and that the same occurred upon enzymatic extraction; this may indicate the presence of proteins bound to carbohydrate molecules, which were transferred to the supernatant (where the protein was concentrated to be isolated).

The mean values found for fiber were statistically different from the control ($p<0.05$), except for the extraction with ascorbic acid (sample 2), meaning that in this method, the fiber content was hardly affected (figure 4). Extraction with NaOH resulted in the highest fiber content of all samples, at 20.32 g/100 g dry basis, indicating that NaOH could not act on the fiber components, but instead, there was a concentration of this content in the final extract.

Samples after extraction with alcalase had the lowest fiber content of (0.70 ± 0.45) g/100 g and showed a significant reduction in fiber content compared to the control sample, which had (9.51 ± 0.57) g/100 g. This can be explained by the action of the enzyme in the hydrolysis of proteins. Chitin is a long-chain polymer of N-acetyl glucosamine present in the insect exoskeleton, where the fiber content is located. Hall et al. [33] verified that the chitin material was successfully removed during hydrolysis and subsequent centrifugation to obtain cricket protein hydrolysates, when using alcalase.

Figure 5 shows the average values obtained for the ash content in each sample, where extractions with NaOH or alcalase presented similar results ($p>0.05$), but were significant ($p<0.05$) different, compared to the control and ascorbic acid extracts. As their values were (9.78 ± 0.77) g/100 g and (8.93 ± 0.78) g/100 g, respectively, both showed higher concentrations compared to the control (4.43 ± 0.03) g/100 g. Amarender et al. [32] also found a higher concentration of minerals in the protein extract using the NaOH method. Makishi et al. [48] also found an increase in mineral content in final protein extracts with the use of NaOH and increased pH. The increase in ash content observed in the NaOH

 doi:10.20944/preprints202411.0190.v1

extract may be attributed to residual sodium hydroxide or its reaction products, such as sodium salts, remaining after the extraction process. Ash content represents the inorganic mineral material of a sample, which can include any residual salts that are left after the treatment. The use of NaOH can also result in the leaching of minerals, either through disruption of the bonds between proteins and these minerals or through the solubilization of inherent mineral components present in the cricket powder [49]. Upon using the enzymatic method, a higher ash content can also be explained by using an alkaline solution (NaOH) to adjust the pH necessary for optimizing enzyme activity during hydrolysis. This concentration of ash content in the enzymatic protein hydrolysate was also observed by Hall et al. [33], with values ranging between 9.5-14.6 % on a dry basis.

Ascorbic acid extraction leaded to the lowest ash content in the protein extract at 1.70 g/100 g. Ascorbic acid is known to have chelating properties, meaning it can bind to metal ions such as iron (Fe^{2+}) and copper (Cu^{2+}) [50]. These minerals are widely found in cricket powders [30, 50], by forming stable complexes with these metal ions, ascorbic acid prevents them from interacting with proteins and other components in the solution. During the extraction process, ascorbic acid can chelate these metal ions, keeping them in the soluble fraction (supernatant), which was separated after centrifugation steps and thus impacting the ash content found in this sample.

Figure 6 shows moisture content values, they are statistically different from each other ($p<0.05$). The values obtained were as expected, given that the final dehydration step was freeze-drying, with the aim of obtaining extracts with a moisture content of less than 5% (w/w). The average values obtained after NaOH, ascorbic acid or enzymatic extractions were (1.26 ± 0.26), (4.55 ± 0.07), and (3.9 ± 0.17), respectively. A lower moisture content, in addition improving preservation and quality of the protein extract, induces an increase in the concentration values of other components [51, 52]. Finally, the moisture content values found in this study were like those found by Hall et al., Amarender et al. and Pellerin & Doyen [32,33,53].

4.3. Yields and extraction rate

The lipid extraction from cricket powder before protein extraction allowed higher protein extraction rates due to an increase in the initial protein concentration. This provides a greater amount of protein material to be extracted throughout the extraction process, resulting in higher yields.

The extraction yields, or the defatting of the initial mass, varied between 69.10-77.81 %, with ascorbic acid extraction having the lowest value and alcalase extraction, the highest. These values were slightly lower than those found by Amarender et al. and Zhao et al. [31, 32]. This can be explained by the modifications made to the lipid extraction method. These lower values may indicate losses in other components, not just lipids. A recent study [54] found lower lipid extraction yields using ethanol compared to hexane, which was more efficient. Additionally, this study also found that ethanol, being an alcohol, could cause protein denaturation and subsequent irreversible aggregation, negatively affecting protein extraction rates. This demonstrates the need for further studies to analyze the structure and profile of protein after the lipid extraction step of cricket powder. Despite the lower yields, ethanol remains a more sustainable and healthier option compared to hexane. Ethanol is produced from renewable resources and has a significantly lower environmental impact, while hexane is derived from non-renewable petroleum sources and poses severe environmental and health risks, including neurotoxicity [54].

The protein extraction rates varied between 56.99 and 85.97%, with the ascorbic acid method having the lowest result as it also had the lowest protein content in the final extract. The enzymatic method had the highest protein extraction rate compared to both chemical methods. This demonstrates the potential for obtaining protein extracts using enzymes although the method used for sample 1 can also be an alternative as NaOH presents economic advantageous as it is cheaper. Similar studies also achieved good results using alcalase to obtain protein extracts with protein contents above 70% dry weight [33,46,55].

 doi:10.20944/preprints202411.0190.v1

Among the samples obtained, enzymatic extraction showed the best results in yield and protein content of the final extract. Physicochemical and functional property analyses are necessary to validate its use in food applications.

5. Conclusions

The results obtained in this study demonstrate that crickets of the species *Acheta domesticus* can serve as an interesting alternative protein source due to their rich nutrient content. The form in which crickets are presented significantly influences consumer acceptance. Insect-derived powders have been identified as one of the best strategies to increase the acceptance level of food products incorporating this new protein source. However, given the limited functionality observed in various studies of product development with insect powder incorporation, other forms have been widely studied to improve this aspect and make this new ingredient appealing to all consumers. The fractionation of protein in insect-derived powders has shown to be one of the potential ways to make this new ingredient functional, more competitive with other protein alternatives, and with potential health benefits (through bioactive compounds already confirmed by scientific literature) as well as environmental benefits.

Among the methods studied in this research, the enzymatic hydrolysis technique achieved the best results, both in terms of the protein content found in the final extract and of the protein extraction yield, which was 85.97%. Lipid extraction prior to protein extraction was effective in removing fat content, thus allowing a greater amount of protein material to be extracted. This step is crucial for contributing to the increase of protein concentration in the final extract. The resulting product also appeared interesting from a functional perspective, because hydrolyzed protein may offer greater digestibility and bioavailability of amino acids. Indeed, the fractionation of protein in insect-derived powders has shown to be one of the potential ways to make this new ingredient functional, more competitive with other protein alternatives, and with potential health benefits, as well as environmental benefits.

Further studies on protein hydrolysates in *Acheta domesticus*, through enzymatic action, are necessary to confirm better functionality. Sensory analyses of these extracts and the development of food products are also important studies to validate this new ingredient in the food industry.

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