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EVALUATION OF PH-MODIFIED CHICKPEA PROTEIN ISOLATE AS A FUNCTIONAL FAT REPLACER IN GERMAN-STYLE COOKED SAUSAGES

The object of research is boiled German-type sausages with reduced fat content. The technological complexity of fat reduction in traditional meat emulsions is a major problem, which inevitably leads to a deterioration in both physicochemical parameters (emulsion stability) and sensory characteristics, such as texture and juiciness. This study was aimed at assessing the ability of chickpea protein isolate (CPI), modified using the pH-adjustment method, to act as a functional fat substitute. At the first stage, a comparison of the techno-functional properties of the modified isolate (solubility, WHC, OHC, EAI and ESI) with those of the native protein was carried out. All functional parameters of CPI were significantly improved by pH-treatment ($p < 0.05$). The solubility increased from 24.33% to 82.67%, and the emulsifying activity index (EAI) from 27.33 to 48.33 m^2/g , which are significant changes for meat systems. For the experiment, modified CPI was introduced at concentrations of 1% (sample CPI1) and 2% (sample CPI2) for partial fat replacement. This was compared with the results of the high-fat control (23%). This combination allowed to significantly ($p < 0.05$) reduce the mass fraction of fat in the finished products. Sample CPI1 showed a decrease of 26.1%, and sample CPI2 showed a decrease of 40.6%. At the same time, the technological yield showed a clear trend towards growth (from 90.67% to 99.00%). Sensory analysis (on a 9-point scale) showed that sample CPI1 (1% CPI) had a sensory profile that was statistically indistinguishable ($p > 0.05$) from the control in all parameters, including taste (8.05 vs. 8.07) and aroma (7.63 vs. 7.78). However, sample CPI2 showed a significant deterioration in organoleptic properties ($p < 0.05$). Thus, pH-modified CPI proved its effectiveness as a fat substitute, and the 1% dosage was identified as the best method for preparing healthy cooked sausages without compromising on taste.

Keywords: chickpea protein isolate, pH adjustment, fat substitute, meat products, traditional sausages, vegetable protein.

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1. Introduction

The global approach to combating non-communicable diseases is putting new demands on the food industry. It is essential to radically change recipes to reduce fat, salt and sugar content [1]. Scientists have found a link between saturated fat intake in red meat and the risk of cardiovascular disease [2]. In this context, the question arises: how to preserve the uniqueness of traditional European meat products while adapting them to modern health standards? Recent studies have shown that the modification of products such as fermented sausages and sauces by adding alternative proteins (derived from plants or invertebrates) is of increasing interest [3, 4]. Therefore, it is relevant to improve the nutritional composition and reduce fat content, while maintaining high sensory characteristics. Animal fat in emulsion sausages is not just about calories. It forms juiciness, texture and a unique flavor and aroma bouquet, which is important for techno-functionality [5]. Inevitably, the quality of the product deteriorates due to mechanical reduction of fat. The only way out is to use functional substitutes. The involvement of plant ingredients, such as legumes and fiber, is quite promising, since it can solve two tasks: reduce fat content and maintain technological parameters at an optimal level [6, 7]. Vegetable proteins are considered

a universal option that meets the requirements of sustainable development and environmental safety [8]. Nevertheless, their successful incorporation into food matrices is a difficult task. The raw material and the processing method significantly affect the bioavailability and functionality of such proteins [9, 10]. Against this background, chickpeas (*Cicer arietinum*) stand out due to their excellent nutritional profile and low allergenicity, especially compared to soybeans [11, 12]. However, the disadvantages of native chickpea protein isolate (CPI), in particular its low solubility and poor gelling and emulsion-forming properties, hinder its widespread use [11].

To unlock the potential of CPI, it is necessary to deliberately modify its structure. pH-adjusting treatment (also known as pH-shifting) is a non-thermal method that changes the protein conformation and improves its functionality [13]. This method (sometimes combined with other physical methods) has been shown to significantly increase the solubility and emulsifying properties of pea proteins [13, 14]. However, the use of pH-modified CPI for fat replacement in German-style meat emulsions remains understudied.

Therefore, the object of the research is German-style cooked sausages with reduced fat content. The aim of the research is to assess the possibility of using pH-modified chickpea protein isolate as an effective

functional fat substitute in the production of cooked Schinkenwurst sausages. The study focused on both the model sausage samples themselves and the modified CPI.

To achieve the aim, the following tasks need to be solved:

1. Conduct a direct comparison of the techno-functional properties of native protein versus pH-modified chickpea protein isolate.
2. Create experimental recipes for cooked Schinkenwurst sausages, where fat will be replaced by 1% and 2% pH-modified isolate, and investigate their nutritional value and technological yield.
3. Using sensory evaluation, determine how different dosages of chickpea protein isolate affect the organoleptic characteristics of finished products.

2. Materials and Methods

2.1. Materials

Pork (ham and shoulder), top grade beef and back fat were purchased from a local certified supplier located in Ukraine. Organic gluten-free chickpea flour TM 'Ahimsa' was used to obtain protein isolate. Nitrite salt (0.6% NaNO₂) and ground black pepper were used as flavoring and salting ingredients, which were purchased from a specialized supplier. All chemical reagents used for analysis were of analytical grade.

2.2. Preparation and modification of chickpea protein isolate (CPI)

CPI was obtained from organic chickpea flour by acid-base extraction. The pH level was controlled using a pH meter N-5170 (Mera-Elwro, Poland). A laboratory centrifuge (MLW, Germany) was used to precipitate proteins. Next, pH-shifting was performed at pH 11 to maximize its functional properties, followed by drying the obtained isolate [15].

2.3. Analysis of functional properties of the isolate

2.3.1. Protein solubility (PS)

A modified method was used to determine solubility by preparing a 1% (w/v) aqueous dispersion of the isolate [16]. The suspension was thoroughly mixed on a MLW magnetic stirrer (Germany) for 30 minutes. Then, it was centrifuged at 4000 g for 20 minutes. The Bradford method was used to determine the amount of protein in the soluble fraction or supernatant using a Spekol 11 spectrophotometer (Carl Zeiss Jena, Germany) [17]. Solubility (PS, %) was determined as the ratio of the amount of protein in the supernatant to the total amount of protein in the original sample.

2.3.2. Water holding capacity (WHC)

A portion of protein (1 g) was weighed into a pre-weighed centrifuge tube, 10 ml of distilled water was added, and the mixture was mixed well on a Thys 2 vibratory shaker (MLW, Germany) for 1 minute. After 30 minutes of hydration, the mixture was centrifuged for 20 minutes at 3000 g. The WHC was calculated as the ratio of grams of water retained to grams of dry protein (g/g) [18].

2.3.3. Oil holding capacity (OHC)

In the process of determining OHC, refined sunflower oil was used instead of distilled water. OHC was determined as grams of oil retained per gram of dry protein [19].

2.3.4. Emulsifying properties

The emulsifying activity index (EAI, m²/g) and emulsion stability index (ESI, min) were determined using the turbidimetric method [20]. A 1% protein solution was prepared in phosphate buffer. The emulsion was created by high-speed homogenization (15 ml of protein solution and 5 ml of refined sunflower oil were processed at 10,000 rpm for 1 min).

After homogenization ($t = 0$) and after 10 minutes ($t = 10$), the optical density was measured spectrophotometrically on a Spekol 11 (Carl Zeiss Jena, Germany) at 500 nm.

2.4. Experimental design and production of cooked sausages

This study aimed to develop a healthier version of the traditional German sausage type Schinkenwurst. Three formulations were developed (Table 1):

- 1) Control;
- 2) CPI1 (1% CPI);
- 3) CPI2 (2% CPI).

Table 1

Recipes of model samples of cooked sausages (g/kg)

Ingredient	Control	CPI1	CPI2
Meat inserts (Einlage)			
Lean pork	400	400	400
Nitrite salt	8	8	8
Ground black pepper	1	1	1
Minced meat emulsion (Grundbrät)			
Lean pork	250	250	250
Top grade beef	100	100	100
Back fat (bacon)	150	112.5	75
Chickpea protein Isolate	–	10	20
Ice water/Ice	91	118.5	146
Total	1000	1000	1000

The minced meat emulsion was prepared by the cutter method. The finished minced meat was filled into cellulose casings with a diameter of 60 mm under vacuum. The formed loaves were subjected to heat treatment in a water bath (Labtech, Ukraine) until a temperature of $72 \pm 1^\circ\text{C}$ was reached in the center of the loaf.

2.5. Nutritional value analysis

The protein content in the sausage samples was analyzed by the Kjeldahl method [21]. The fat content was determined by Soxhlet extraction [22]. The moisture content was determined by drying in a WS 100 drying oven (MLW, Germany) at 105°C , and the ash content by combustion in a muffle furnace LM 312.11 (MLW, Germany) at 550°C according to standard methods [23]. The energy value (kcal/100 g) was determined directly based on the protein and fat content using generally accepted coefficients.

2.6. Yield of the finished product

The yield of the finished product was calculated as the ratio of the weight of the sausage loaf after heat treatment and cooling to the weight of the raw loaf before heat treatment, weighing was carried out on an analytical balance Nagma AV IV S/3 (Nagma, Germany). The result was expressed in percentage (%).

2.7. Sensory analysis

The sensory evaluation was carried out in accordance with the general requirements of ISO 6658:2017. To detail the product profile, the Scorecard method was used using a 9-point scale developed according to the principles of sensory analysis [24]. A group of ten trained tasters was involved in the work to evaluate the appearance, aroma, taste, aftertaste and texture.

2.8. Statistical analysis

All measurements were performed in triplicate ($n = 3$). The results are presented as the mean \pm standard deviation. One-way analysis of

variance (ANOVA) with Tukey's test was used to detect statistically significant differences ($p < 0.05$). R software was used for statistical analysis of data (4.5.1).

3. Results and Discussion

3.1. Functional properties of pH-modified chickpea protein isolate (CPI)

The first step was to evaluate how the pH-adjusting treatment affected the main functional properties of CPI; these properties were important for its role in meat emulsion. Table 2 contains a comparative analysis of the native (CPI) and pH-modified (PHCPI) isolates.

According to the data presented in Table 2, it is obvious that the pH adjustment procedure is effective. The modified protein (PHCPI) showed significantly better results in all studied indicators ($p < 0.05$). The solubility increased most significantly (by 3.4 times) from 24.33% to 82.67%. This result is important, since solubility is the basis for other functional properties. It is well known that extreme pH values cause structural changes, in particular, controlled unfolding of protein globules. This, in turn, allows access to hydrophobic and polar groups that were previously hidden [11, 13]. The emulsifying activity index (EAI) increased by 77%, and the emulsion stability (ESI) increased more than twice (by 2.2 times). Such dynamics clearly indicate the mechanism of action: the modified protein, due to increased surface hydrophobicity, is more actively adsorbed at the oil-water interface and forms a mechanically more stable protein film [13]. WHC and OHC also significantly ($p < 0.05$) increased, which is crucial for ensuring juiciness and high yield of the finished product [7]. Due to low solubility (24.33%) and low emulsifying activity (27.33 m²/g), native chickpea protein isolate has limited technological potential (Table 2). The use of proteins with such characteristics in emulsified meat systems leads to a deterioration in organoleptic parameters and prevents the formation of a stable protein matrix. For this reason, for further modeling of minced meat systems, only the pH-modified isolate was selected, which demonstrated the required level of functionality. The effectiveness of this isolate was evaluated in comparison with the traditional high-fat recipe, which does not meet the requirements of a healthy diet.

3.2. Nutritional value and yield of the finished product

After confirming the high CPI functionality of, the next step was to evaluate its effect directly in the meat system. The nutritional value and technological yield of the finished sausages were analyzed in comparison with the high-fat control. The obtained data are given in Table 3.

A detailed analysis of the nutritional value (Table 3) proved the effectiveness of the selected optimization strategy. In particular, in samples CPI1 and CPI2, a significant ($p < 0.05$) decrease in fat content was recorded compared to the control. As a result, there was a natural decrease in calorie content, which was our strategic goal in the context of healthy nutrition [1, 2]. As for the mineral composition, the expected increase in ash content was obtained in samples CPI1 and CPI2 ($p < 0.05$). This fact is a direct consequence of the nature of the introduced component, since chickpea protein is much richer in minerals compared to animal fat [12]. From a technological point of view, the results are even more revealing: the yield of the finished product showed a clear upward trend (growth from 90.67% to 99.00%). This numerical trend is not accidental – it directly correlates with the high WHC of the modified protein (Table 2) and confirms its role as an effective moisture stabilizer in the meat matrix during heat treatment [7].

3.3. Sensory evaluation of cooked sausages

Let's consider this stage as crucial for the entire experiment. The logic is obvious, since even the most advanced technological advantages lose their meaning if the final product does not find a response from the consumer. Detailed results of sensory profiling are summarized in Table 4.

The final verdict on the success of any reformulation is made by the consumer, therefore it is possible to consider the sensory analysis data (Table 4) as a critical indicator of the viability of the development [1, 4]. Sample CPI1 (1% CPI) demonstrated, without exaggeration, the effect of "organoleptic mimicry": tasters did not detect a statistically significant difference ($p > 0.05$) between it and the control sample for any of the key descriptors. This is a breakthrough moment for plant ingredients. This result proves that the pH-modified isolate works as a structurally active but sensory neutral agent at this concentration, completely avoiding the formation of a specific "bean" profile, which is often a stumbling block for technologists [11].

Table 2

Functional properties of native and pH-modified chickpea protein isolate

Property	Native CPI	pH-modified CPI (PHCPI)
Solubility (<i>PS</i>), %	24.33 ± 2.08 ^b	82.67 ± 6.51 ^a
Water holding capacity (<i>WHC</i>), g/g	2.77 ± 0.06 ^b	4.20 ± 0.40 ^a
Oil holding capacity (<i>OHC</i>), g/g	3.00 ± 0.20 ^b	3.97 ± 0.42 ^a
Emulsifying activity index (<i>EAI</i>), m ² /g	27.33 ± 1.53 ^b	48.33 ± 4.04 ^a
Emulsion stability index (<i>ESI</i>), min	29.33 ± 1.15 ^b	63.33 ± 1.53 ^a

Note: different letters of the Latin alphabet (superscript) within one line indicate a statistically significant difference ($p < 0.05$)

Table 3

Nutritional value and yield of the finished product of cooked sausage samples

Indicator	Control	CPI1 (1% CPI)	CPI2 (2% CPI)
Moisture, %	57.00 ± 4.36 ^a	62.33 ± 2.08 ^a	62.67 ± 1.53 ^a
Protein, %	15.00 ± 1.00 ^a	16.33 ± 0.58 ^a	16.33 ± 1.15 ^a
Fat, %	23.00 ± 1.73 ^a	17.00 ± 1.73 ^b	13.67 ± 1.53 ^b
Ash, %	2.27 ± 0.06 ^b	2.57 ± 0.15 ^a	2.77 ± 0.06 ^a
Energy value, kcal/100 g	267.00 ^a	218.32 ^b	188.35 ^c
Product yield, %	90.67 ± 3.06 ^a	93.33 ± 5.69 ^a	99.00 ± 2.65 ^a

Note: different letters of the Latin alphabet (superscript) within one line indicate a statistically significant difference ($p < 0.05$)

Table 4

Results of sensory evaluation of cooked sausages

Indicator	Control	CPI1 (1% CPI)	CPI2 (2% CPI)
Appearance	7.88 ± 0.53 ^a	8.07 ± 0.53 ^a	7.68 ± 0.43 ^a
Aroma	7.78 ± 0.49 ^a	7.63 ± 0.55 ^a	6.72 ± 0.53 ^b
Taste	8.07 ± 0.40 ^a	8.05 ± 0.55 ^a	7.13 ± 0.46 ^b
Aftertaste	7.78 ± 0.46 ^a	7.49 ± 0.54 ^a	6.78 ± 0.40 ^b
Texture	7.66 ± 0.54 ^a	8.01 ± 0.47 ^a	7.84 ± 0.58 ^a

Note: different letters of the Latin alphabet (superscript) within the same line indicate a statistically significant difference ($p < 0.05$)

As the dosage increases, the situation changes. The CPI2 sample (2% CPI), although it remained within the acceptable range, still crossed the sensory threshold, showing a significant decrease in the scores in the aroma, taste and aftertaste categories ($p < 0.05$). However, the texture analysis revealed an intriguing feature. A clear trend is found towards improved tactile sensations in the CPI samples (score 8.01 for CPI1). However, no statistical differences were found ($p > 0.05$). This indicates that the modified protein not only fills the structure, but also effectively reproduces the enveloping effect of fat, preventing the "rubbery" consistency typical of low-calorie products [6]. CPI1 actually hit the "golden mean" – we got a product with a significantly improved composition (26% less fat), which does not differ from the traditional high-fat standard in terms of sensory. Despite the significant decrease in fat content in the CPI1 sample (Table 3), the texture score was 8.01 points, which is statistically not different from the control (Table 4). This indicates that the lack of fat was fully compensated by the preliminary modification of the protein, which proves its functional properties in cooked sausage.

3.4. Discussion

The addition of protein isolate resulted in an increase in the technological yield of cooked sausages. This allows to consider the result obtained from the addition of a functional ingredient as a pragmatic resource efficiency, in the context of global challenges to food systems [25]. The task of replacing animal fats in cooked sausage formulations [26] is mainly an engineering one. The study highlights the basic principle: the success of the final product depends on the functionality of the raw material. The improvement of the emulsifying and water-holding properties of CPI, which was achieved by pH-treatment, is an example of smart food system design. This strategy has a significant advantage compared to other methods of microstructural modification already applied to animal proteins (e. g. whey [27]). In contrast to allergenic dairy derivatives, pH-modified chickpea isolate provides a powerful, yet hypoallergenic, technological tool. This confirms the transformation of modified CPI from a simple "alternative" to a sophisticated tool for modern food architecture [15].

Although the results of the work are clear and statistically significant, it is important to understand that it has some limitations. The research was primarily conducted in laboratory and pilot conditions. Although the equipment was of industrial type, real mass production processes can change the stability of the emulsion and the final product yield. In addition, the analysis was based only on physicochemical and sensory characteristics. The CPI effect on storage stability parameters, in particular on the course of oxidative processes (TBARS), color stability and microbiological safety, was not investigated. Finally, the sensory evaluation was carried out with the participation of a small trained expert panel ($n = 10$). This method is ideal for detecting differences in the profile, but it cannot fully predict the final consumer perception. Purchase intentions are influenced by a wider range of hedonic, cultural and economic factors [28].

It has been confirmed that pH-modified CPI has proven to be a highly effective structurant with direct application potential. The fact

that only 1% of the additive allows for a 26.1% fat reduction without any sensory compromises provides manufacturers with a ready-made universal technological solution for the "clean label" and "better-for-you" segments. It is critical to visualize the mechanics of CPI integration into the protein-fat matrix using rheology methods and confocal laser scanning microscopy (CLSM). Furthermore, the success in cooked emulsions opens up the space to test this ingredient in more demanding systems: low-fat pâtés, restructured hams or even in the acidic environment of fermented sausages.

4. Conclusions

1. The obtained data show that pH-correcting treatment as a modification method is effective. It was found that the techno-functional profile of chickpea protein compared to the native form was significantly improved ($p < 0.05$). An increase in solubility by 3.4 times (up to 82.67%), an increase in the emulsifying activity index by 77% and an increase in water-holding capacity by 52% are examples of the dynamics of indicators. This allows reclassifying CPI from a simple filler to a class of ingredients with high functionality.

2. The study was aimed at developing optimized recipes for cooked Schinkenwurst sausages. Replacing animal fat with 1% (CPI1) and 2% (CPI2) of the modified isolate was a strategy that allowed integrating the plant component into the traditional meat matrix. As a result of the optimization of the nutrient profile, the fat content decreased by 26.1% and 40.6%, respectively ($p < 0.05$). The replacement of ingredients improved the technological parameters, as a clear correlation was found between the reduction in caloric content and the increase in the yield of the finished product (from 90.67% to 99.00%).

3. Sensory analysis allowed to determine the acceptable limits of intervention. The CPI1 sample (1%) was determined as the rational optimum, since it showed a profile (Taste 8.05) statistically identical to the high-fat control ($p > 0.05$). At the same time, it was found that the concentration of 2% already exceeds the threshold of sensory perception. This led to a significant decrease in hedonic assessments of taste and aroma ($p < 0.05$). Thus, 1% is a compromise point between consumer properties and technological feasibility.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Manuscript has no associated data.

Use of artificial intelligence

In accordance with the editorial policy, the authors provide detailed information about the use of AI:

- ChatGPT (GPT-4o model from OpenAI);
- the tool was used in Sections 1, 2, and 3 of this manuscript.
- the tool was used exclusively for technical proofreading: checking grammar, spelling, and punctuation, which is consistent with the permitted tasks according to the journal's policy. No stylistic changes, adaptation of scientific style, sentence shortening, or content generation using AI were performed.

The authors performed a full manual review of each proposed technical correction to ensure that the corrections did not affect the content or accuracy of scientific terminology.

The use of the tool to correct grammatical errors in no way affected the data obtained, their interpretation, results, or conclusions of research, which are entirely the authors' own.

Declaration submitted by: Olha Vasylenko.

Authors contributions

Iryna Kurmakova: Conceptualization, Methodology, Resources, Supervision, Project administration, Writing – review and editing; **Nadiia Lapytska:** Conceptualization, Methodology, Resources, Supervision, Writing – review and editing; **Hanna Novik:** Conceptualization, Methodology, Resources, Visualization, Writing – original draft, Writing – review and editing; **Olena Bondar:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review and editing; **Olha Vasylenko:** Supervision, Formal analysis, Investigation, Resources, Data curation, Visualization, Project administration, Writing – original draft, Writing – review and editing; **Tetiana Holovko:** Conceptualization, Methodology, Resources, Supervision, Project administration, Writing – review and editing; **Maksym Zhrebkin:** Conceptualization, Methodology, Resources, Writing – review and editing; **Iryna Levchenko:** Investigation, Resources, Data curation, Writing – review and editing; **Oleh Starynskyi:** Investigation, Resources, Data curation, Writing – review and editing.

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