DOI: 10.22630/TPFP.2024.1.10021

ORIGINAL PAPER
Received: 08.10.2024
Accepted: 14.01.2025

POTENTIAL BENEFITS OF INSECT PROTEIN IN PREVENTING OVERWEIGHT AND OBESITY

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Abstract: Obesity in children and adolescents is one of the most serious health challenges of the 21st century, and the number of overweight and obese people is steadily increasing. In recent years, entomophagy, or the consumption of insects, has been proposed as an alternative source of protein with economic and environmental advantages over traditional meat production. Edible insects have high nutritional value. They are rich in protein, recommended fats, vitamins, and minerals. Moreover, numerous animal studies show insect protein can slow weight gain, improve the immune response, reduce inflammation, and benefit energy metabolism. Despite the challenges of promoting and accepting insect protein, its health and environmental benefits may make it an essential part of a balanced diet in the future. Including insects in the diet can help overcome obesity-related health problems, potentially help control weight and reduce the risk of obesity-related diseases. However, further research, including clinical trials, is needed to confirm the health benefits of insect protein. Educational campaigns can help break down cultural barriers and increase the acceptance of entomophagy.

Key words: edible insects, entomophagy, insect protein, nutritional value of insects, obesity, health benefits of insects, neophobia

INTRODUCTION

Obesity and overweight among children and adolescents are one of the most serious health challenges of the 21st century. According to the World Health Organization (WHO), the number of overweight and obese children and adolescents is on a clear upward trend. In 1975, the number of obese children was 11 million, while in 2016, it had risen to 124 million. In Poland, the problem affects about 10% of young children (1–3 years), 30% of early school-age children, and almost 22% of adolescents up to age 15 [1]. The increasing rate of overweight and obesity among children represents a major public health challenge, influencing the development of numerous physical and mental illnesses in childhood, which can persist into adulthood. Obesity increases the incidence of comorbidities, such as stroke, atherosclerosis, myocardial infarction, cardiovascular disease, diabetes, hypertension, and hyperlipidaemia [2].

Various obesity treatment regimens have been proposed, including physical activity, appropriate diet, and medication to aid weight reduction. Most known anti-obesity drugs suppress the appetite, reducing the absorption of components or energy sources, i.e., sugars or fats, and are often accompanied by adverse side effects [3]. It is, therefore, extremely important to step up research to identify natural products that offer safe effects to support therapy in the fight against being overweight. According to Sanyaolu et al. [4], obesity in childhood and adolescence is caused by a mismatch between energy intake and expenditure, regardless of genetic factors.

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The daily diet's amount and composition of macronutrients (carbohydrates, fats, and proteins) determines the total energy expenditure. Proteins provide less energy than an equivalent amount of fats, and their percentage contribution in a healthy, balanced diet is around 20%. In comparison, carbohydrates contribute 50% and fats 30% [5]. It is also important to remember that daily protein intake in childhood is essential for normal growth and development and varies with age, sex and physical activity [6]. As shown in a study in mice [7], when diets are low in protein energy, a mechanism known as protein leverage stimulates a compensatory increase in food intake to achieve a specific absolute protein intake. Saner et al. [8] showed that small changes in protein intake may have been a major contributor to the obesity epidemic in the USA between 1961 and 2013. A recent study by Larsen et al. [9] found that high-protein and low-glycaemic-index diets were more effective in maintaining weight loss and improving metabolic parameters than low-protein diets. In a study conducted by Saner et al. [8] on a group of obese children and adolescents aged six to eighteen years, it was found that changing the amount of protein in the diet could help reduce the total energy intake in obese adolescents. Hall's (2019) population-based study [10], which examined trends in macronutrient composition over the past four decades in the United States, found a decrease in the percentage of energy intake from protein sources and an increase in total energy intake, increasing BMI. One reason for this phenomenon is the increasing popularity of highly processed foods, which contain only 9.5% of energy from protein sources [13]. According to Hall [10], the consumption of large amounts of highly processed foods is a major cause of the growing obesity epidemic. As shown by Saner et al. [8], only a balanced macronutrient composition in the diet of children and adolescents can help combat the problem of obesity.

When discussing obesity, it is also worth mentioning the use of the satiety index [11] and the effect of individual nutrients on the body's satiety level, i.e. the feeling of fullness after a meal. A diet based on this index can be an alternative to restrictive diets. Most studies on the effect of protein on satiety have found that protein has a more significant effect on satiety than carbohydrates or fats. Current data suggest that increasing the protein intake plays a key role in satiety [12].

Given the various proposals for using currently available foods, it is worth considering edible insects in the diet. On the one hand, they can be a potential source of high-quality protein [13, 14, 15, 16], and on the other hand, they can be an effective component of low-carbohydrate diets [17, 18, 19]. Of all food components, protein and dietary fibre [20] have the strongest satiating properties. Protein is thought to release neurochemicals that suppress appetite [21]. It is hypothesised that insect protein-induced satiety is mediated by the release of specific neurochemicals and hormones that regulate appetite and food intake. Key neurochemicals involved in this process include serotonin, dopamine, cholecystokinin (CCK), and glucagon-like peptide-1 (GLP-1). The mechanisms by which insect protein induces satiety include stimulating these hormones and neurochemicals. Insect protein ingestion triggers the release of CCK and GLP-1, which send signals to the brain to promote satiety and reduce appetite. In addition, insect protein can affect the brain's reward system by increasing dopamine levels, increasing satiety and reducing food intake. These mechanisms suggest insect protein may effectively regulate appetite and control weight [22, 23]. A high-protein meal increases the likelihood of reducing the energy content of the next meal by up to 25%. In addition, carbohydrate reduction may also have a positive effect on satiety, as carbohydrates have been cited as the ingredient with the lowest satiety potential [24, 25, 26]. Skotnicka et al. [21], based on a study of insect food intake in 71 volunteers, found that pancakes with 30% flour from *Alphitobius diaperinus* and those with 20% and 30% flour from the house cricket (*Acheta domesticus*) were the most satiating. They concluded that protein content had the greatest effect on inducing satiety. The results support the hypothesis that insect-based foods could be an ingredient in diets for treating obesity, for carbohydrate-restricted diets, and as alternative protein sources [26].

Insects have been valued as a food source in many cultures for centuries. Although the topic has gained renewed attention recently, Meyer-Rochow [27] proposed the consumption of insects as an alternative food and feed source nearly 50 years ago. It is currently estimated that over 2 billion people regularly consume insects [28].

Insect protein offers several environmental benefits, making it a sustainable alternative to traditional meat production. Firstly, insect farming requires significantly less water compared to conventional livestock farming. For instance, producing 1 kg of insect protein requires about 1 litre of water, whereas producing the same amount of beef protein requires approximately 22,000 litres. This drastic reduction in water usage makes insect farming a more sustainable option. Insect farming also needs less land than traditional livestock farming. Insects can be farmed in vertical systems, which allows for efficient use of space [15, 16, 24, 28]. This means that insect farming can be conducted in urban areas and other locations where space is limited, further contributing to its sustainability. Insect production also generates significantly fewer greenhouse gases compared to traditional meat production. For example, insect farming's methane and nitrous oxide emissions are much lower than cattle farming emissions

[28]. This reduction in greenhouse gas emissions helps mitigate climate change and reduces the environmental footprint of food production. Moreover, insects can be fed organic waste, reducing waste and improving resource efficiency. For example, Housefly larvae (*Musca domestica*) can be fed kitchen waste, converting it into valuable protein. This reduces the amount of waste that ends up in landfills and creates a sustainable source of protein. Insects have a high feed conversion efficiency, meaning they convert a more significant proportion of their feed into protein [15, 24, 28]. This efficiency makes insect farming a more resource-effective way to produce protein.

However, the introduction of insects for human consumption has met with resistance, particularly in Europe, mainly due to cultural issues. While cultural issues are indeed relevant to the low consumption of insects in Europe, they are not the only challenge. Legislative issues are also a significant barrier. The European Union has strict rules for approving and marketing novel foods, including insects. The Novel Foods Regulation (EU) 2015/2283 requires extensive safety assessments and authorisation procedures before insects can be marketed as food. While ensuring consumer safety, this regulatory framework can be time-consuming and costly for producers, limiting the availability and diversity of insect-based products on the market [15, 16]. In addition, there is a lack of harmonisation of regulations across European countries, which can create additional barriers for producers wishing to enter multiple markets. For example, some countries may have more lenient regulations while others impose stricter controls, leading to inconsistencies and confusion in the industry [19]. In conclusion, while cultural issues are important, regulatory challenges also play a key role in the low consumption of insects in Europe. Tackling cultural and regulatory barriers is therefore essential to increase the acceptance and consumption of insect-based foods.

Edible insects have great potential as a food source. Their sustainability, nutritional value, economic viability, and low environmental impact make them attractive for addressing global food security and sustainability challenges. This review aims to synthesise and critically evaluate the current knowledge on the potential use of insect protein in the prevention of overweight and obesity, and to identify gaps in the literature that require further research.

NUTRITIONAL VALUE OF EDIBLE INSECTS

The reasons for promoting insects as a source of nutrients in the diet are increasingly supported by a growing body of scientific research. The number of publications on entomophagy increased from 16 in 2001–2010 to 49 in 2011–2015, and 50 articles were published between 2016 and September 2017. Given this trend, it is reasonable to estimate that over 200 studies have been published from 2014 to 2024 [29].

Current research mainly focuses on edible insect species approved for consumption. This is due to food safety considerations and legal regulations determining which insect species can be used as food. The most commonly studied species in the scientific literature are *Tenebrio molitor*, *Acheta domesticus*, *Hermetia illucens, Bombyx mori, Imbrasia belina* and *Rhynchophorus phoenicis* [30, 31].

Edible insects are rich in protein and beneficial fats, vitamins and minerals, making them a valuable dietary component. Compared to traditional sources of protein, such as meat, insects can provide protein with high biological value at a lower calorie load (Tab. 1). According to Zeng & Li (2021) [40], a higher protein intake, including insect-derived protein, can affect the composition of the gut microbiome, which in turn can affect metabolic health and weight control. The average amount of protein in edible insects ranges from 10% to 70% of dry weight, which is higher than some plant products such as soya and lentils [24]. For example, the protein content of fresh edible butterfly caterpillars and chicken meat is 28 g/100 g and 21 g/100 g fresh weight, respectively [32]. The wide range of protein content in edible insects is due to the huge number of species and their diversity, developmental stages (larvae, pupae, adult insects), culture conditions, processing methods and analytical methods (Tab. 1). The content of essential amino acids varies depending on the species and developmental stage of the insect. It ranges from 46% to 96% of the total amino acids [32].

Insect protein contains all 20 amino acids, fully meeting the adult human requirement for these components. Insect protein is a good source of valine, histidine, phenylalanine and tyrosine, and in some species also tryptophan, lysine and threonine, although in varying amounts depending on the species [40]. According to Malla et al. [41], the tryptophan content ranges from 0.5% to 1.5%, and according to a study published in the International Journal of Biological Macromolecules, the amino acid profile of insects may be deficient in it [42]. The amino acid indices in edible insects such as *Tenebrio molitor* and *Alphitobius diaperinus* are comparable to those found in soybeans and bovine casein [43]. An undoubtedly important feature is insect protein's digestibility, which is comparable to milk, soya, or casein, ranging from 54% for *Tenebrio molitor* to as high as 98% for some South American insect species [40]. On average, these values are similar to those reported for egg white (95%) or beef (98%), and higher than many plant proteins [44].

Table 1. Chemical composition, nutritional value and vitamin content of selected edible insects and conventional protein sources.

Insect species and conventional protein sources	Protein (%)	Fat (%)	Carbohydrates (%)	Fibre (%)	Vitamins and minerals (mg/100g)	Ref.
Tenebrio molitor	47.0	30.0	6.0	5.0	Fe: 5.0, Zn: 10.0, Ca: 20.0, Vit B12: 1.2, Vit A: 0.1	[30,26]
Hermetia illucens	42.0	35.0	7.0	6.0	Fe: 4.5, Zn: 8.0, Ca: 18.0, Vit B12: 2.3, Vit A: 0.2	[30,26]
Acheta domesticus	70.0	12.0	5.0	2.0	Fe: 3.0, Zn: 9.0, Ca: 15.0, Vit B12: 5.4, Vit A: 0.3	[30,26]
Bombyx mori	55.0	28.0	8.0	4.0	Fe: 6.0, Zn: 11.0, Ca: 22.0, Vit B12: 3.1, Vit A: 0.2	[30,26]
Schistocerca gregaria	62.0	17.0	10.0	3.0	Fe: 4.0, Zn: 7.0, Ca: 19.0, Vit B12: 1.8, Vit A: 0.1	[30,26]
Alphitobius diaperinus	60.0	18.0	6.0	5.0	Fe: 4.0, Zn: 8.0, Ca: 16.0, Vit B12: 2.0, Vit A: 0.2	[33,26]
Locusta migratoria	50.4	19.6	4.8	15.7	P: 29.6, Ca: 2.2, Vit B12: 1.5, Vit A: 0.1	[34,26]
Macrotermes nigeriensis	20.4	36.8	18.8	15.2	Fe: 95.6, Zn: 4.2, Ca: 20.0, Vit B12: 0.9, Vit A: 0.1	[35,26]
Gryllus bimaculatus	60.0	23.0	5.0	2.0	Fe: 3.0, Zn: 9.0, Ca: 15.0, Vit B12: 4.0, Vit A: 0.2	[36,26]
Musca domestica	57.0	25.0	6.0	4.0	Fe: 5.0, Zn: 10.0, Ca: 18.0, Vit B12: 2.5, Vit A: 0.2	[37,26]
Protaetia brevitarsis	63.0	15.0	8.0	5.0	Fe: 4.5, Zn: 8.0, Ca: 17.0, Vit B12: 3.0, Vit A: 0.2	[38,26]
Chicken	27.0	14.0	0.0	0.0	Fe: 1.3, Zn: 1.0, Ca: 11.0, Vit B12: 0.6, Vit A: 0.0	[31,39]
Beef	26.0	20.0	0.0	0.0	Fe: 2.6, Zn: 4.0, Ca: 12.0, Vit B12: 2.5, Vit A: 0.0	[31,39]
Soybeans	36.0	20.0	30.0	9.0	Fe: 15.7, Zn: 4.9, Ca: 277.0, Vit B12: 0.0, Vit A: 0.0	[31,39]
Eggs	13.0	11.0	1.0	0.0	Fe: 1.2, Zn: 1.0, Ca: 50.0, Vit B12: 1.1, Vit A: 0.3	[31,39]
Fish (salmon)	20.0	13.0	0.0	0.0	Fe: 0.8, Zn: 0.6, Ca: 20.0, Vit B12: 3.2, Vit A: 0.1	[31,39]

Source: own elaboration based on [31, 39].

The fat content of insects ranges from 8 to 70% based on dry weight and varies considerably depending on the species and stage of development. Butterfly larvae (Lepidoptera) and bugs (Heteroptera), have a significantly higher fat content than other edible insects. In contrast, adult insects, compared to their larvae or juvenile forms, are characterised by a fat content of less than 20% [45]. The fatty acid composition is comparable to that of poultry and fish fat. It is characterised by a relatively high content of C18 fatty acids, including oleic, linoleic and linolenic acids. It is high in many species and high in saturated fatty acids, especially palmitic acid [44]. Mature insects are particularly valued for their rich content of polyunsaturated fatty acids, exceeding traditional sources such as pork and beef. Insects belonging to the order Orthoptera, such as the *Locusta migratoria*, are an exceptional source of linoleic acid. On the other hand, butterfly larvae have a particularly high content of α-linolenic acid [45]. The higher fat content of butterfly and bug larvae is related to their energy requirements during growth and metamorphosis. In contrast, adult insects have lower fat levels because their main purpose is reproduction and flight [46].

Insects are not a rich source of carbohydrates. Their average content ranges from 6.71% in bugs to 15.98% in cicadas, and is mainly found in the form of chitin, which is classified as a polysaccharide. As an exoskeleton component, chitin is structurally similar to cellulose [47]. The content of chitin in different insect species can vary considerably and it can be digestible by humans, thus having nutritional and health benefits beyond improving gut health as a prebiotic [48]. Oliveira et al. [49] showed that chitin and its derivative, chitosan, exhibit fibre properties that can act as prebiotics [50], selectively stimulating the growth and activity of beneficial bacteria in the colon. In insects, chitin forms a complex mainly with proteins and lipids, affecting their digestibility [49]. Chitin, a linear polymer consisting of N-acetylglucosamine units, and their derivative, chitosan, formed by the deacetylation of chitin and containing a higher number of glucosamine units, exhibit important fibre properties. The deacetylation process increases the solubility of chitosan in acidic solutions and gives it a higher positive charge, which affects its ability to bind lipids in the human gastrointestinal tract [51].

Insects are also a source of micronutrients such as copper, iron, magnesium, manganese, phosphorus, selenium and zinc. According to Zhou et al. [26], the Orthoptera and Coleoptera species contain higher levels of chemically active calcium, copper, magnesium, manganese, and zinc than beef, with crickets, in particular, having higher iron bioavailability. Some studies have confirmed that insect consumption can provide a high percentage of humans' daily recommended mineral intake, particularly for iron [49]. *Macrotermes nigeriensis*, a genus of termites that is consumed in many parts of the world, especially in Africa, Asia, Australia and Latin America [52], contains 95.6 mg of iron per 100 g of dry weight [49], which is much higher than the iron content of pork loin on the bone (only 1 mg/100 g), veal shoulder (2.9 mg/100 g) or pork liver (18.7 mg/100 g) [53]. Termites are also a popular food source in Kenya, especially in rural regions. Research has shown that about 80% of respondents in Kenya have tasted termites, and 55% regularly buy termite food products such as dried and roasted ones. Termite consumption in Kenya can range from a few times a month to several times a week, depending on availability and culinary traditions [54].

Insects are a rich source of both water-soluble and fat-soluble vitamins, including significant amounts of vitamin B12 (Tab. 1) For example, the vitamin B12 content ranges from 1.08 µg/100 g in whiteflies to 13.2 µg/100 g dry weight in cockroaches (Blattodea) [40, 55].

OVERWEIGHT AND OBESITY-RELATED HEALTH EFFECTS OF CONSUMPTION OF SELECTED INSECT SPECIES

The solution to obesity and overweight lies not only in reducing calorie intake but also in improving the quality of the diet. As mentioned at the beginning of this article, the high protein content of insects promotes satiety, which helps to reduce the overall food intake and control weight. Unlike the fats found in meat products, insects are also a valuable source of unsaturated fatty acids. They are also low in carbohydrates, which, when consumed in excess, can lead to weight gain and metabolic disorders. The nutritional profile of insects supports muscle maintenance and growth, which is essential for metabolic health and effective weight management [45].

Numerous studies have shown (Tab. 2) that certain insect-derived substances regulate lipid metabolism and blood lipid levels, with anti-obesity effects. In vivo studies using *Tenebrio molitor* larvae have shown that they reduce lipid accumulation and triglyceride levels, leading to weight loss in obese mice [26]. Daily administration of *Tenebrio molitor* larvae powder to obese mice fed a high-fat diet inhibited weight gain and reduced visceral fat [56]. The powder also reduced hepatic steatosis and decreased plasma levels of liver enzymes such as alanine aminotransferase and aspartate aminotransferase, which are indicators of liver damage.

Table 2. Health effects of consumption of selected insect species in the context of overweight and obesity

Insect species	Health effects related to overweight and obesity	Ref.
Tenebrio molitor	Reduced lipid accumulation, reduced triglyceride levels, improved blood lipid profile	[57]
Hermetia illucens	Reduced body weight, reduced visceral fat accumulation, improved lipid metabolism	[58]
Acheta domesticus	Increased feeling of satiety, improved lipid profile, lowered blood glucose levels	[59]
Bombyx mori	Regulated lipid metabolic processes, reduced fat accumulation, improved insulin sensitivity	[60]
Schistocerca gregaria	Lowered cholesterol levels, improved lipid profile, reduced body weight	[61]
Alphitobius diaperinus	Reduced fat accumulation, improved lipid metabolism, reduced blood glucose levels	[62]
Locusta migratoria	Reduced body weight, improved lipid profile, lowered blood glucose levels	[63]
Macrotermes nigeriensis	Reduced fat accumulation, improved lipid metabolism, reduced blood glucose levels	[64]
Gryllus bimaculatus	Increased feeling of satiety, improved lipid profile, lowered blood glucose levels	[65]
Musca domestica	Reduced fat accumulation, improved lipid metabolism, lowered blood glucose levels	[66]
Protaetia brevitarsis	Reduced body weight, improved lipid profile, lowered blood glucose levels	[67]

Source: own elaboration based on [57, 58, 59, 60, 61, 62, 63, 64].

A study by Park et al. [68] confirmed these observations. Using *Tenebrio molitor* larvae powder in the diet of mice, the authors reported reductions in body weight, subcutaneous and visceral fat volume, hepatic adipocyte accumulation, adipocyte size, leptin levels, levels of adipogenesis-related genes and plasma concentrations of total cholesterol, triglycerides and glucose. In another in vivo study in spontaneously hypertensive rats fed a diet based on *Tenebrio molitor*, a slight reduction in fat mass was observed [69]. The reduction in visceral fat improved hepatic insulin action and reduced the expression of inflammatory cytokines.

Allomyrina dichotoma larvae extract reduced the serum triglyceride and leptin levels in obese mice and reduced body weight gain, organ weights and adipose tissue volume in a dose-dependent manner [70]. In a study in a rat model fed a high-fat diet, it was observed that ethanolic extracts from *Gryllus bimaculatus* exhibited anti-obesity properties. Rats that received the extract for two months showed a reduction in fat mass, especially abdominal fat [71]. The peptides of the mulberry silkworm pupa have hypolipidemic effects, regulating lipid metabolic processes in the body, inhibiting the formation of fat in preadipocytes and reducing lipid accumulation and adipocyte size, which helps to combat obesity [72].

Other studies have shown that protein hydrolysate from silkworm larvae increases glucose uptake and reduces fat accumulation by increasing leptin expression [72]. Ethanolic extract of *Oxya chinensis* locusts slows carbohydrate digestion and glucose uptake by inhibiting the activity of carbohydrate digestive enzymes, thereby reducing postprandial hyperglycaemia induced by dietary carbohydrates [67]. It has been observed in mice that a protein-enriched extract of housefly *Musca domestica* larvae reduces plasma triglyceride, total cholesterol and low-density lipoprotein levels while increasing high-density lipoprotein levels [56].

Feeding rats a meal containing various amounts of house fly pupae reduced visceral fat [73]. These effects were probably stimulated by the chitin, monounsaturated fatty acids, and bioactive compounds in house fly pupae that have not been identified yet [73].

Chitosan isolated from *Acheta domesticus* has been shown to retain lipids. Its lipid-binding capacity is comparable to shrimp chitosan [74], which has been shown to exert anti-obesity effects by controlling body weight in pigs [46].

It has also been reported that cricket ethanol extract reduced body weight, intestinal fat and total cholesterol in mice given a high-fat diet for 14 weeks [75]. A study by Huang et al. [76] confirmed a significant reduction in food intake and fat accumulation in obese rats fed chitin, which prevented weight gain and significantly reduced serum lipids such as total cholesterol, triglycerides and low-density cholesterol.

Administration of *Protaetia brevitarsis* larvae extract to mice with high-fat diet-induced obesity reduced body weight, liver adipocyte count, and serum lipid levels [56]. A high-fat cricket-based diet in rats had beneficial effects on lipid metabolism and body fat content [51].

Recent studies have continued to explore innovative solutions to this growing problem. For instance, Rizou et al. (2022) [78] demonstrated that probiotic supplementation in the diet of Tenebrio molitor larvae significantly improved their nutritional profile, increasing the protein content and reducing fat levels – factors that may enhance their potential as a functional food ingredient in metabolic health. Additionally, Dreassi et al. (2017) [79] showed that the fatty acid composition of *T. molitor* can be modulated through dietary interventions, improving the balance of polyunsaturated fatty acids, which are known to support lipid metabolism and reduce inflammation. These findings support the use of mealworm protein as a promising dietary component in obesity prevention and lipid profile improvement. Furthermore, Kang et al. (2023) [62] demonstrated that the inclusion of *Tenebrio molitor* and *Alphitobius diaperinus* in a high-fat diet significantly altered the gut microbiota composition in obese mice. These changes were associated with improved metabolic parameters, including reduced body weight gain and enhanced lipid metabolism. This study highlights the potential of insect-based diets to modulate host metabolism through microbiota-dependent mechanisms.

The few human clinical studies on the consumption of edible insects provide valuable information on their impact on human health [29, 80]. These have shown, among other things, that the consumption of insect protein can lead to increased post-meal amino acid levels, suggesting its high bioavailability and potential as an alternative protein source. In addition, studies have shown that insect consumption can improve gut health by increasing gut microbiota diversity and reducing inflammation. They further confirmed that supplementation with chitosan, a chitin derivative, can lead to weight loss and improved lipid profile, which is important for preventing obesity and metabolic diseases. Finally, studies on insect protein intake have shown that it can improve metabolic health indicators such as blood glucose levels and lipid profile, suggesting its potential health benefits.

ADVOCATING INSECT PROTEIN AS A SUSTAINABLE AND EFFECTIVE STRATEGY FOR TREATING OVERWEIGHT AND OBESITY

Insect protein is increasingly seen as a good alternative and sustainable source. Despite the growing interest in edible insects, their promotion as a viable solution to combat obesity faces several challenges, especially in European countries. One of the main problems is public acceptance and poor promotion of the potential of edible insect-based products [81]. Convincing consumers to change their eating habits takes time and education. Information and education campaigns can help to break down cultural barriers and increase the acceptance of insect protein. This acceptance of insect-based foods among children, the next generation of consumers, is particularly under-researched, as most studies have targeted adults.

Previous consumer research in European countries shows a low acceptance of edible insect-based products. Most of these studies have been conducted in the Netherlands, Belgium and several other northern countries, where insect-based products have become commercially available in recent years [81, 82, 83]. The acceptance of insect-based foods is largely determined by food neophobia, which is the tendency to avoid unfamiliar or new foods [84]. Food neophobia plays a key role in accepting insects as food and the willingness to eat insect-based foods [86].

A study by Ainslee et al. [87] of 181 school-aged children found that Danish children showed a moderate willingness to try insect-based foods, suggesting potential in this segment of the population. In a study by Dupont and Fiebelkorn [88] on the attitudes and acceptance of young people from Germany towards the consumption of insects and farmed meat, the authors noted that of the children and adolescents who took part in the study, 70.9% had already heard that insects could be used as food and knew what this meant, while 22.4% had heard of the possibility of eating insects, but did not know exactly what this meant. Only 6.7% did not know that insects could be eaten. Of the respondents, 17.8% had eaten insects once and 5.3% several times. Only 0.1% stated that they regularly ate insects. In addition, the study showed that the respondents were more likely to eat burgers with lab-grown meat than burgers with insects.

Three focus group interviews conducted with children aged 4–5 years in a public kindergarten in Sweden concluded that using children's imagination and curiosity about new things, experimenting with insect-based products and ingredients in familiar dishes and discussing different ways of eating insects can increase children's acceptance of eating insects [89].

A study by Marquis [89] discussed how exposure and familiarisation with edible insects can influence adolescents' attitudes towards entomophagy. The activity, which involved 662 Canadian high school students, was shown to have a significant positive impact on the participants' acceptance of edible insects. These results can be used in promotional activities to accelerate the acceptance of edible insects. A study by Florence et al. [90] found that people were more likely to consume food products containing insects than whole insects, and the youngest were the most likely to consume insects. A study on the effect of educational interventions on attitudes and the willingness to consume insect-based foods was conducted by Szczepanski et al. [91]. After an educational class on 'Entomophagy and sustainability', the authors reported a slight increase in the willingness to consume insect-based foods. Attitudes appeared to be the strongest predictor of willingness to consume, while knowledge had no significant effect. The type of insect, the order of tasting, and children's food neophobia had a significant effect on food acceptance.

Szlachciuk and Żakowska-Biemans [92] studied the perception, beliefs, and willingness to consume insects among Polish consumers. The study included 1,000 respondents and found that dietary neophobia was a key factor influencing beliefs and perceptions of the consumption of insects and insect protein products. Respondents were more likely to consume insect protein products than visible insects. Sogari et al. [93] examined the relationship between the willingness to try insects and food neophobia among young adults in Italy. The study included 88 participants and found that males were more open to trying insects than females, and food neophobia was negatively correlated with a willingness to eat insects. Kinyuru et al. [94] studied the influence of food neophobia and socio-cultural factors on the consumption of edible insects in Uganda. The study included three insect species: locusts, African termites, and wingless termites. The results indicated that food neophobia and socio-cultural factors significantly impacted consumers' willingness to consume insects.

CONCLUSIONS AND FUTURE PERSPECTIVES

Obesity and overweight in children and adolescents is one of the most serious health challenges of the 21st century, and the number of overweight and obese people is steadily increasing, according to the World Health Organization (WHO). In the search for sustainable solutions, entomophagy has been proposed as an alternative protein source with economic and environmental advantages over traditional meat production. Insect protein is increasingly being explored as a potential tool in the fight against obesity.

Edible insects such as crickets, mealworm larvae and grasshoppers are characterised by high levels of complete protein, low carbohydrate contents and the presence of beneficial nutrients such as unsaturated fatty acids, vitamins, minerals, and non-digestible polysaccharides like chitin. Numerous studies suggest insect protein can effectively control body weight by increasing satiety and postprandial thermogenesis.

However, the introduction of insect protein into the diet faces several challenges, including social and cultural acceptance. Despite growing interest, edible insect-based products are still not very popular in European countries. Education and information campaigns are key to breaking down barriers and increasing consumer acceptance of insect protein, especially among more receptive consumers such as children and teenagers.

In conclusion, insect protein has the potential to be a valuable tool in the fight against obesity and overweight. However, its full potential requires further research, especially clinical studies, and educational and promotional activities in primary, secondary, and higher education. Increasing consumer acceptance of insects as sustainable food is key to promoting the regular consumption of insect-based foods.

REFERENCES

[1] **Taraszewska A. 2018.** Nadwaga i otyłości wśród dzieci i młodzież. Narodowe Centrum Edukacji Żywieniowej. Narodowe Centrum Edukacji Żywieniowej. (https://ncez.pzh.gov.pl/abc-zywienia/nadwaga-i-otylosc-wsrod-dzieci-i-mlodziezy/, access: 10.12.1024).

[2] **Pakhare M., Anjankar A. 2024.** Critical Correlation Between Obesity and Cardiovascular Diseases and Recent Advancements in Obesity. Cureus 4(16): 1:e51681. https://www.doi.org/10.7759/cureus.51681

[3] **Tak Y.J., Lee S.Y. 2021.** Anti-Obesity Drugs: Long-Term Efficacy and Safety: An Updated Review. World J Mens Health 39(2): 208–221. https://www.doi.org/10.5534/wjmh.200010

[4] **Sanyaolu A., Okorie C., Qi X., Locke J., Rehman S., Patidar R. 2019.** Childhood and Adolescent Obesity in the United States: A Public Health Concern. Global Pediatric Health 6: 2333794X19891305. https://www.doi.org/10.1177/2333794X19891305

[5] **Jarosz. M, Rychlik E., Cichocka A. 2019.** Białkowska M. Czy wiesz ile potrzebujesz energii z pożywienia? Narodowe Centrum Edukacji Żywieniowej. (https://ncez.pzh.gov.pl/wp-content/uploads/2021/03/broszura-energia-1.pdf, access: 14.11.2024).

[6] **Phillips S.M., Fulgoni V.L. 2022.** 3rd: Assessment of the Protein Intake of Americans: NHANES 2003–2014. Nutrients 15, 14(6): 1234. https://www.doi.org/10.3390/nu14061234

[7] **Sørensen A., Mayntz D., Raubenheimer D., Simpson S.J. 2023.** Effects of Dietary Protein to Carbohydrate Balance on Energy Intake and Body Adiposity in Mice. Obesity (Silver Spring) 31(1): 45–56.

[8] **Saner C., Tassoni D., Harcourt B.E., Kao K.T., Alexander E.J., McCallum Z., Olds T., Rowlands A.V., Burgner D.P., Simpson S.J., Raubenheimer D., Senior A.M., Juonala M., Sabin M.A. 2020.** Evidence for Protein Leverage in Children and Adolescents with Obesity. Obesity 28: 822–829. https://www.doi.org/10.1002/oby.22755

[9] **Larsen T.M., Dalskov S.M., van Baak M., Jebb S.A., Papadaki A., Pfeiffer A.F., Martinez J.A., Handjieva-Darlenska T., Kunesova M., Holst C., Saris W.H., Astrup A. 2023.** Diets with High or Low Protein Content and Glycemic Index for Weight-Loss Maintenance. New England Journal of Medicine 384(22): 2102–2113.

[10] **Hall K.D. 2019.** The potential role of protein leverage in the US obesity epidemic. Obesity (Silver Spring) 27: 1222–1224.

[11] **Barakat G.M., Ramadan W., Assi G., El Khoury N.B. 2024.** Satiety: a gut–brain–relationship. The Journal of Physiological Sciences 74: 17, 74(1):11. https://www.doi.org/10.1186/s12576-024-00904-9

[12] **Moon J., Koh G. 2020.** Clinical Evidence and Mechanisms of High-Protein Diet-Induced Weight Loss. Journal of Obesity & Metabolic Syndrome 30, 29(3): 166–173. https://www.doi.org/10.7570/jomes20028

[13] **Skotnicka M., Karwowska K., Kłobukowski F., Borkowska A., Pieszko M. 2021.** Possibilities of the Development of Edible Insect-Based Foods in Europe. Foods 10, 766.

[14] **Villaseñor V.M., Enriquez-Vara J.N., Urías-Silva J.E., Mojica L. 2021.** Edible Insects: Techno-functional Properties Food and Feed Applications and Biological Potential. Food Reviews International 38(1): 866–892. https://doi.org/10.1080/87559129.2021.1890116

[15] **Van Huis A., Van Itterbeeck J., Klunder H., Mertens E., Halloran A., Muir G., Vantomme P. 2020.** Edible insects: Future prospects for food and feed security. Food and Agriculture Organization of the United Nations, Rome.

[16] **Mancini S., Moruzzo R. 2021.** Insects as food and feed: A review of potential benefits and risks. Journal of Insects as Food and Feed 7(5): 477–492.

[17] **Di Rosa C., Lattanzi G., Taylor S.F., Manfrini S., Khazrai Y.M. 2020.** Very low calorie ketogenic diets in overweight and obesity treatment: Effects on anthropometric parameters, body composition, satiety, lipid profile and microbiota. Obesity Research & Clinical Practice 14: 491–503.

[18] **Loper H., Leine M., Bassoff L., Sample J., Romero-Ortega M., Gustafson K.J., Taylor D.M., Schiefer M.A. 2021.** Both high fat and high carbohydrate diets impair vagus nerve signaling of satiety. Scientific Reports 11: 10394.

[19] **Dobermann D., Swift J.A., Field L.M. 2017.** Opportunities and hurdles of edible insects for food and feed. Nutrition Bulletin 42: 293–308.

[20] **Martini D., Brusamolino A., Del Bo' C., Laureati M., Porrini M., Riso P. 2018.** Effect of fiber and protein-enriched pasta formulations on satiety-related sensations and afternoon snacking in Italian healthy female subjects. Physiology & Behavior 185: 61–69.

[21] **Skotnicka M., Mazurek A., Karwowska K., Folwarski M. 2022.** Satiety of Edible Insect-Based Food Products as a Component of Body Weight Control. Nutrients 14, 2147. https://www.doi.org/10.3390/nu14102147

[22] **Zhang Y., Wang D., Zhou S., Duan H., Guo J., Yan W. 2022.** Effects of Dietary Protein to Carbohydrate Balance on Energy Intake and Body Adiposity in Mice. Obesity (Silver Spring) 31(1): 45–56.

[23] **Aguilar-Toalá J.E., Cruz-Monterrosa R.G., Liceaga A.M. 2022.** Beyond human consumption of edible insects: Health benefits and safety issues. Insects 13(11): 1007. https://www.doi.org/10.3390/insects13111007

[24] **Schlüter O., Rumpold B., Holzhauser T., Roth A., Vogel R.F., Quasigroch W., Vogel S., Heinz V., Jäger H., Bandick N., Kulling S., Knorr D., Steinberg P., Engel K.H. 2017.** Safety aspects of the production of foods and food ingredients from insects. Molecular Nutrition & Food Research 61(6): 1600520. https://www.doi.org/10.1002/mnfr.20160052

[25] **Bayham B.E., Greenway F.L., Johnson W.D., Dhurandhar N.V. 2014.** A randomized trial to manipulate the quality instead of quantity of dietary proteins to influence the markers of satiety. Journal of Diabetes and its Complications 28: 547–552.
[26] **Zhou Y., Wang D., Zhou S., Duan H., Guo J., Yan W. 2022.** Nutritional composition, health benefits and functional value of edible insects: A review. Foods 11(24): 3961. https://www.doi.org/10.3390/foods11243961
[27] **Meyer-Rochow V.B. 2017.** Therapeutic arthropods and other, largely terrestrial, folk-medicinally important invertebrates: a comparative survey and review. Journal of Ethnobiology and Ethnomedicine 13: 9.
[28] **Gantner M., Piesik D. 2023.** Jadalne, pożyteczne i zrównoważone – owady jako alternatywne źródło białka. In: Gutkowska. (ed.), Partnerstwo instytucjonalne w kształtowaniu zachowań żywieniowych w trosce o zdrowie publiczne. Wydawnictwo SGGW, Warszawa: 510–525.
[29] **Cunha N., Andrade V., Ruivo P., Pinto P. 2023.** Effects of Insect Consumption on Human Health: A Systematic Review of Human Studies. Nutrients 15(14): 3076. https://www.doi.org/10.3390/nu15143076.
[30] **Tang C., Yang D., Liao H., Sun H., Liu C., Wei L., Li F. 2019.** Edible insects as a food source: a review. Food Production, Processing and Nutrition 1: 8.
[31] **Li M., Mao C., Li X., Jiang L., Zhang W., Li M., Liu H., Fang Y., Liu S., Yang G., Hou X. 2023.** Edible Insects: A New Sustainable Nutritional Resource Worth Promoting. Foods 12(22): 4073.
[32] Ordoñez-Araque R., Quishpillo-Miranda N., Ramos-Guerrero L. 2022. Edible Insects for Humans and Animals: Nutritional Composition and an Option for Mitigating Environmental Damage. Insects 13: 944. https://www.doi.org/10.3390/insects13100944
[33] **Kurečka M., Kulma M., Petříčková D., Plachý V., Kouřimská L. 2021.** Larvae and pupae of Alphitobius diaperinus as promising protein alternatives. European Food Research and Technology 247: 2527–2532.
[34] **Mohamed E.H.A. 2020.** Determination of Nutritive Value of the Edible migratory locust Locusta migratoria. International Journal of Advance**s in** Pharmacy, Biology and Chemistry, 9(2): 1–6.
[35] **Paiko Y.B., Azeh Y., Ibrahim I.L., Awwal I. 2018.** Nutritional composition of Edible Termites (Macrotermes bellicosus) Consumed in Paikoro Local Government, Niger State, Nigeria. Lapai Journal of Applied and Natural Sciences, 3(1): 214–221.
[36] **Udomsil N., Imsoonthornruksa S., Gosalawit C., Ketudat-Cairns M. 2019.** Nutritional Values and Functional Properties of House Cricket (Acheta domesticus) and Field Cricket (Gryllus bimaculatus). Food Science and Technology Research 25(4): 597–605.
[37] **Conceição da-Silva W., Silva É.B.R. da, Silva J.A.R. da et al. 2024.** Nutritional Value of the Larvae of the Black Soldier Fly (Hermetia illucens) and the House Fly (Musca domestica) as a Food Alternative for Farm Animals – A Systematic Review. Insects 15(8): 619.
[38] **Cho E.C., Ahn S., Hwang H.J., Shin K.O., Kim S., Choi Y.J. 2024.** Investigating the Nutritional and Functional Properties of Protaetia brevitarsis Larvae and Isolated Soy Protein Mixtures as Alternative Protein Sources. Foods 13(10): 1540.
[39] **Ajomiwe N., Boland M., Phongthai S., Bagiyal M., Singh J., Kaur L. 2024.** Protein Nutrition: Understanding Structure, Digestibility, and Bioavailability for Optimal Health. Foods 13(11): 1771.
[40] **Rumpold B.A., Schlüter O.K. 2019.**Nutritional composition and safety aspects of edible insects. Molecular Nutrition & Food Research 63(1): 1800111.
[41] **Malla N., Nørgaard J.V., Roos N. 2023.** Protein quality of edible insects in the view of current assessment methods. Animal Frontiers 13(4): 50–63. https://www.doi.org/10.1093/af/vfad015.
[42] **Zhao X., Vázquez-Gutiérrez J.L., Johansson D.P., Landberg R., Langton M. 2024.** Insects as a sustainable protein source: A review of recent research. International Journal of Biological Macromolecules 183: 1–10.
[43] **Perez-Santaescolastica C., de Pril I., van de Voorde I., Fraeye I. 2023.** Fatty Acid and Amino Acid Profiles of Seven Edible Insects: Focus on Lipid Class Composition and Protein Conversion Factors. Foods 12(22): 4090. https://www.doi.org/10.3390/foods12224090
[44] **Kourimská L., Adámková A. 2016.** Nutritional and sensory quality of edible insects. NFS Journal 4: 22–26. https://www.doi.org/10.1016/j.nfs.2016.07.001
[45] **Lisboa H.M., Nascimento A., Arruda A., Sarinho A., Lima J., Batista L., Dantas M.F., Andrade R. 2024.** Unlocking the Potential of Insect-Based Proteins: Sustainable Solutions for Global Food Security and Nutrition. Foods 13: 1846. https://www.doi.org/10.3390/foods13121846.
[46] **Sinclair B.J., Marshall K.E. 2018.** The many roles of fats in overwintering insects. Journal of Experimental Biology 221(1): jeb161836. https://www.doi.org/10.1242/jeb.161836
[47] **Lima D.B., Almeida R.D., Pasquali M., Borges S.P. 2018.** Fook ML, Lisboa HM. Physical characterization and modeling of chitosan/peg blends for injectable scaffolds. Carbohydrate Polymers 189: 238–249.
[48] **Ojha S., Bekhit A.E.D., Grune T., Schlüter O.K. 2021**. Bioavailability of nutrients from edible insects. Current Opinion In Food Science 41: 240–248.
[49] **Oliveira L.A., S.M. Santana Pereira, Dias A.K., da Silva Paes S., Grancieri M., Jimenez L.G.S, Piler de Carvalho C.W., de Oliveira E.E., Stampini Duarte Martino H., Della Lucia C.M. 2024.** Nutritional content, amino acid profile,

and protein properties of edible insects (Tenebrio molitor and Gryllus assimilis) powders at different stages of development. Journal of Food Composition and Analysis 125: 105804. https://www.doi.org/10.1016/j.jfca.2023.105804.

[50] **Gibson G.R., Hutkins R., Sanders M.E., Prescott S.L., Reimer R.A., Salminen S.J., Scott K., Stanton C., Swanson K.S., Cani P.D., Verbeke K., Reid G. 2017**. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology 14(8): 491–502. https://www.doi.org/10.1038/nrgastro.2017.75

[51] **Bonin M., Irion A.L., Jürß A., Pascual S., Cord-Landwehr S., Planas A., Moerschbacher B.M. 2024.** Engineering of a chitin deacetylase to generate tailor-made chitosan polymers. PLOS Biology 22(1): e3002459. https://doi.org/10.1371/journal.pbio.3002459

[52] **Adepoju O.T., Omotayo O.O. 2014.** Nutrient composition of four species of winged termites consumed in western Kenya. Journal of Food Composition and Analysis 33(1): 1–6. https://www.doi.org/10.1016/j.jfca.2013.11.004

[53] **Kunachowicz H., Nadolna I., Przygoda B., Iwanow K. 2005.** Tabele składu i wartości odżywczej żywności. PZWL, Warsaw.

[54] **Alemu M.H., Olsen S.B. 2018.** Kenyan Consumers' Experience of Using Edible Insects as Food and Their Preferences for Selected Insect-Based Food Products. in: Edible Insects in Sustainable Food Systems, Springer 22: 363–374.

[55] **Schmidt A., Call L.M., Macheiner L., Mayer H.K. 2019.** Determination of vitamin B12 in four edible insect species by immunoaffinity and ultra-high performance liquid chromatography. Food Chemistry 281: 124–129.

[56] **Seo M., Goo T.V., Chung M.Y., Baek M., Hwang J.-S., Kim M.-A., Yun E.-Y. 2017.** Tenebrio molitor larvae inhibit adipogenesis through AMPK and MAPKs signaling in 3T3-L1 adipocytes and obesity in high-fat diet-induced obese mice. International Journal of Molecular Sciences 18(3): 518. https://doi.org/10.3390/ijms18030518

[57] **Park B.M., Lim H.J., Lee B.J. 2021.** Anti-obesity effects of Tenebrio molitor larvae powder in high-fat diet-induced obese mice. Journal of Nutrition and Health 54(4): 342–354.

[58] **Barragan-Fonseca K.B., Dicke M., van Loon J.J.A. 2017.** Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. Wageningen Academic Publishers, Wageningen.

[59] **Rosas-Campos R., Meza-Rios A., Rodriguez-Sanabria J.S., De la Rosa-Bibiano R., Corona-Cervantes K., García--Mena J., Santos A., Sandoval-Rodriguez A. 2022.** Dietary supplementation with Mexican foods, Opuntia ficus indica, Theobroma cacao, and Acheta domesticus: Improving obesogenic and microbiota features in obese mice. Frontiers in Nutrition 9: 987222.

[60] **Kim M.W., Ham Y.J., Kim H.B., Lee J.Y., Lim J.D., Lee H.T. 2023.** Anti-obesity effects of the larval powder of steamed and lyophilized mature silkworms in a newly designed adult mouse model. Foods 12(19): 3613.

[61] **Mmbone S. 2013.** Effects of diet on the nutritional composition of the desert locust Schistocerca gregaria (Orthoptera: *Acrididae*). African Journal of Tropical Entomology Research 2(1): 1–10. https://doi.org/10.58697/AJTER020101

[62] **Kang Y., Oba P.M., Gaulke C.A., Sánchez-Sánchez L., Swanson K.S. 2023.** Dietary inclusion of yellow mealworms (*Tenebrio molitor*) and lesser mealworms (*Alphitobius diaperinus*) modifies intestinal microbiota populations of diet-induced obesity mice. Journal of Nutrition 153(11): 3220–3236.

[63] **Salama S.M. 2020.** Nutrient composition and bioactive components of the migratory locust (*Locusta migratoria*). In: A. Mariod (ed.), African Edible Insects As Alternative Source of Food, Oil, Protein and Bioactive Components. Springer, Cham: 231–239.

[64] **Igwe C.U., Onwuliri V.A., Ojiako A.O., Arukwe J.U. 2011.** Effects of Macrotermes nigeriensis based diet on hepatic and serum lipids of albino rats. Australian Journal of Basic and Applied Sciences 5(7): 906–910.

[65] **Kim M.H., Kim S.J. 2023.** Gryllus bimaculatus-containing diets protect against dexamethasone-induced muscle atrophy, but not high-fat diet-induced obesity. Food Supplements and Biomaterials for Health 1(4): e41.

[66] **Boatta F., van Hal J., Beukeboom L.W., Ellers J. 2024.** High-fat and high-sugar diets induce rapid adaptations of fat storage in the house fly *Musca domestica* L. Journal of Evolutionary Biology 37(11): 1368–1377.

[67] **Park K., Jung S., Ha J.H., Jeong Y. 2025.** Protaetia brevitarsis Hydrolysate Mitigates Muscle Dysfunction and Ectopic Fat Deposition Triggered by a High-Fat Diet in Mice. Nutrients 17(2): 213. https://www.doi.org/10.3390/nu17020213

[68] **Park J.E., Han J.S. 2020.** Oxya chinensis sinuosa extract: Potent glycosidase inhibitor alleviates postprandial hyperglycemia in diabetic mice. Journal of Life Science 30: 1054–1062.

[69] **Pessina F., Frosini M., Marcolongo P., Fusi F., Saponara S., Gamberucci A., Valoti M., Giustarini D., Fiorenzani P., Gorelli B., Francardi V., Botta M., Dreassi E. 2020.** Antihypertensive, cardio- and neuro-protective effects of Tenebrio molitor (Coleoptera: Tenebrionidae) defatted larvae in spontaneously hypertensive rats. PLOS ONE 15(5): e0233788. https://doi.org/10.1371/journal.pone.0233788

[70] **Yoon Y.I., Chung M.Y., Hwang J.S., Han M.S, Goo T.W., Yun E.Y. 2015.** Allomyrina dichotoma (Arthropoda: Insecta) larvae confer resistance to obesity in mice fed a high-fat diet. Nutrients 7: 1978–1991.

[71] **Ahn M.Y., Hwang J.S., Kim M.J., Park K.K. 2016.** Antilipidemic effects and gene expression profiling of the glycosaminoglycans from cricket in rats on a high fat diet. Archives of Pharmacal Research 39: 926–936.

[72] **Ryu S.P. 2014.** Silkworm pupae powder ingestion increases fat metabolism in swim-trained rats. The Journal of Nutritional Biochemistry 18: 141–149.

[73] **Ido A., Iwai T., Ito K., Ohta T., Mizushige T., Kishida T., Miura C., Miura T. 2015.** Dietary effects of housefly (Musca domestica) (Diptera: Muscidae) pupae on the growth performance and the resistance against bacterial pathogen in red sea bream (Pagrus major) (Perciformes: Sparidae). Applied Entomology and Zoology 50: 213–221. https://doi.org/10.1007/s13355-015-0325-z
[74] **Malm M., Liceaga A.M. 2021.** Physicochemical properties of chitosan from two commonly reared edible cricket species, and its application as a hypolipidemic and antimicrobial agent, Polysaccharides 2: 339–353. https://www.doi.org/10.3390/polysaccharides2020022
[75] **Im A.R., Yang W.K., Park Y.C., Kim S.H., Chae S. 2018.** Hepatoprotective effects of insect extracts in an animal model of nonalcoholic fatty liver disease. Nutrients 10(6): 735. https://doi.org/10.3390/nu10060735
[76] **Huang J., Wu Q., Lin Z., Liu S., Su Q., Pan Y. 2020.** Therapeutic effects of chitin from Pleurotus eryngii on high-fat diet induced obesity in rats. Acta Scientiarum Polonorum Technologia Alimentaria19, 3: 279–289.
[77] **Otto C., Lane M.D. 2005.** Adipose development: from stem cell to adipocyte. Critical Reviews in Biochemistry and Molecular Biology 40: 229–242. https://www.doi.org/10.1080/10409230591008189.
[78] **Rizou E., Kalogiouri N., Bisba M., Papadimitriou A., Kyrila G., Lazou A., Andreadis S., Hatzikamari M., Mourtzinos I., Touraki M. 2022.** Amelioration of growth, nutritional value, and microbial load of *Tenebrio molitor* through probiotic supplemented feed. European Food Research and Technology 248: 727–739. https://doi.org/10.1007/s00217-021-03925-5
[79] **Dreassi E., Cito A., Zanfini A., Materozzi L., Botta M., Francardi V. 2017.** Dietary fatty acids influence the growth and fatty acid composition of the yellow mealworm Tenebrio molitor (Coleoptera: Tenebrionidae). Lipids 52: 285–294. https://doi.org/10.1007/s11745-016-4220-3
[80] **Ros-Baró M., Casas-Agustench P., Díaz-Rizzolo D.A., Batlle-Bayer L., Adrià-Acosta F., Aguilar-Martínez A., Medina F.X., Pujolà M., Bach-Faig A. 2022.** Edible Insect Consumption for Human and Planetary Health: A Systematic Review. International Journal of Environmental Research and Public Health 19(18): 11653. https://www.doi.org/10.3390/ijerph191811653.
[81] **Mancini S., Sogari G., Menozzi D., Nuvoloni R., Torracca B., Moruzzo R., Paci G. 2019.** Factors predicting the intention of eating an insect-based product. Foods 8(7): 1–13.
[82] **Van Thielen L., Vermuyten S., Storms B., Rumpold B., Van Campenhout L. 2018.** Consumer acceptance of foods containing edible insects in Belgium two years after their introduction to the market. Journal of Insects as Food and Feed 1: 1–10.
[83] **Caparros Megido R., Haubruge É., Francis F. 2018.** Insects, The Next European Foodie Craze? In: A. Halloran, R. Flore, P. Vantomme, N. Roos (eds), Edible Insects in Sustainable Food Systems. Springer, Cham: 353–361.
[84] **Deroy O., Reade B., Spence C. 2015.** The insectivore's dilemma, and how to take the West out of it. Food Quality and Preference 44: 44–55.
[85] **La Barbera F., Verneau F., Amato M., Grunert K. 2018.** Understanding Westerners' disgust for the eating of insects: The role of food neophobia and implicit associations. Food Quality and Preference 64: 120–125. https://www.doi.org/10.1016/j.foodqual.2017.10.002
[87] **Ainslee L., Erhard A.L., Silva M.S., Damsbo-Svendsen M., Bat-El Menadeva Karpantschof B., Sørensen H., Frøst M.B. 2023.** Acceptance of insect foods among Danish children: Effects of information provision, food neophobia, disgust sensitivity, and species on willingness to try. Food Quality and Preference 104: 713. https://www.doi.org/10.1016/j.foodqual.2022.104713
[88] **Dupont J., Fiebelkorn F. 2020.** Attitudes and acceptance of young people toward the consumption of insects and cultured meat in Germany. Food Quality and Preference 85: 103983. https://www.doi.org/10.1016/j.foodqual.2020.103983.
[89] **Nyberg M., Olsson V., Wendin K. 2021.** 'Would you like to eat an insect?' – Children's perceptions of and thoughts about eating insects. International Journal of Consumer Studies 45: 248–258. https://www.doi.org/10.1111/ijcs.12616.
[89] **Marquis D. 2023.** Targeting adolescents as agents of change for an entomophageous future. British Educational Research Journal 49: 1161–1186. https://www.doi.org/10.1002/berj.3891.
[90] **Florença S.G., Guiné R.P.F., Gonçalves F.J.A, Barroca M.J, Ferreir M., Costa C.A., Correia P.M.R., Cardoso A.P., Campos S., Anjos O. et al. 2022.** The Motivations for Consumption of Edible Insects: A Systematic Review. Foods 11: 3643. https://www.doi.org/.3390/foods11223643.
[91] **Szczepanski L., Dupont J., Schade F., Hellberg H., Büscher M., Fiebelkorn F. 2022.** Effectiveness of a teaching unit on the willingness to consume insect-based food – An intervention study with adolescents from Germany. Frontiers in Nutrition 9: 889805. https://www.doi.org/10.3389/fnut.2022.889805.
[92] **Szlachciuk J., Żakowska-Biemans S. 2024.** Breaking the Taboo: Understanding the Relationship between Perception, Beliefs, Willingness to Eat Insects, and Food Neophobia among Polish Adults. Foods 13(6): 944.
[93] **Sogari G., Menozzi D., Mora C. 2018.** The food neophobia scale and young adults' intention to eat insect products. International Journal of Consumer Studies 42(6): 715–723.
[94] **Kinyuru J.N., Konyole S.O., Kenji G.M., Onyango C.A., Owino V.O., Owuor B.O., Estambale B.B., Friis H., Roos N. 2020.** Insects as food: Illuminating the food neophobia and socio-cultural factors influencing consumption and consumers' willingness to consume three edible insects in Uganda. Journal of Insects as Food and Feed 6(1): 1–10.

DOI: 10.22630/TPFP.2024.1.10019

ORIGINAL PAPER
Received: 15.10.2024
Accepted: 08.12.2024

ASSESSMENT OF NUTRITIONAL KNOWLEDGE AMONG PARENTS OF 6-YEAR-OLD CHILDREN: FOOD ALLERGIES AND INTOLERANCES

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Abstract: Correct nutrition in early childhood plays an important role in the development of the body as the intensive process of physical and cognitive development continues. During this period, the parents are primarily responsible for meeting nutritional requirements. However, this challenge is complicated by the increasing incidence of food allergies and intolerances, which affect approximately 8% of children. The aim of this study was to assess parents' knowledge of nutrition, with a particular focus on food allergies and intolerances. The study was conducted using an electronic questionnaire that included socio-demographic questions about general and specific nutritional knowledge and food allergies and intolerances. The responses of 240 parents of children aged six years who answered all the questions were included in the analysis. The analysis showed that 71% of parents had very good general nutritional knowledge. However, parents of children with allergies showed a significantly more detailed understanding of nutrition, especially in areas related to allergen identification. Compared to parents of healthy children, they were also better at selecting allergen-free foods. At the same time, the results of the study reveal significant gaps in the nutritional knowledge of parents of healthy children, highlighting the need for nutritional education in this population group.

Key words: nutritional knowledge, allergies, intolerances, children

INTRODUCTION

Proper nutrition in early childhood plays an important role in the appropriate development of the body, as this is a time of intense physical, cognitive and emotional development. A balanced diet supports the proper development of the, i.a., immune, nervous and motor systems, which has a direct impact on a child's health in both the short and long term [1, 2]. During this time, the primary responsibility for meeting a child's nutritional requirements lies with the parents, who must provide healthy meals that contain all the essential nutrients. However, this challenge is becoming more complicated as the prevalence of allergies and food intolerances has increased in recent years, particularly among children [3, 4].

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Food allergies are estimated to affect approx. 8% of children and 2% of adults worldwide, and lactose and gluten intolerance are also common dietary restrictions in many regions [5]. These conditions require stricter dietary adherence and constant vigilance, especially by parents [6]. These allergies can severely limit food choices, so parents need to have sufficient knowledge about allergen elimination and be able to introduce safe alternative foods that provide their child with all the necessary nutrients. In addition, the growing awareness of the risks associated with food allergies and intolerances is forcing parents to plan their diets in greater detail, which can be time-consuming but is crucial for the child's proper development [7]. As a result, these parents often have a higher level of nutritional knowledge than parents of children without dietary restrictions [8].

However, there is evidence that parents of children with food allergies may also have nutritional knowledge gaps. While these parents may be able to avoid allergens, they may lack the knowledge to ensure that their children are getting all the nutrients they need, particularly when dietary restrictions limit the intake of important food groups [9]. Research suggests that such gaps in nutritional knowledge can have long-term consequences, as children of parents with limited nutritional understanding may be more likely to develop poor eating habits or nutrient deficiencies [10].

The number of gluten- and lactose-free products available has increased in recent years, while widespread discussion of intolerances has contributed to greater public awareness of nutrition [11]. It is now easier for parents of children with food allergies and intolerances to learn how to manage these conditions. However, popular-science sources can contain misinformation that is not supported by scientific evidence. As a result, misconceptions are common, with many adults believing that allergen-free products are healthier and should be included in the diet, even when there is no medical justification for doing so [12].

Given the above information and the importance of the role of parents in the proper nutrition of their children, the aim of this study was to assess their nutritional knowledge, particularly in relation to food allergies and intolerances.

MATERIAL AND METHODS

A cross-sectional study was conducted using a questionnaire prepared in Google Forms and shared on social media in open groups of public and private kindergartens. Inclusion criteria for the study were having a child aged 6 years and a parental declaration of no food allergies and intolerances or a food allergy and/or food intolerance diagnosed by an allergist. Exclusion criteria for the study were having a child younger or older than 6 years of age; reporting a food allergy and/or intolerance but not confirmed by an allergist; not answering all questions about knowledge of food allergies and intolerances; incomplete or incorrectly completed questionnaires. The age group (6 years) was chosen because this is the age at which children start school, and it is also the transition from early to middle childhood, which can have a significant impact on eating habits and allergies [13]. Furthermore, the choice of one age group ensured consistency and limited variability due to different developmental stages.

The sample for the study was selected using the voluntary response sampling method, which resulted in the collection of 318 questionnaires. However, this did not provide a representative sample of the study population. Of the total questionnaires collected, 240 questionnaires meeting the inclusion and exclusion criteria were used for further analyses (Fig. 1). Of these, 102 were from parents of children with confirmed food allergies or intolerances (group A+I), and 138 were from parents of healthy children (group non-(A+I)).

Data were collected using a self-administered questionnaire. It was designed in the Department of Human Nutrition (WULS-SGGW) for a survey of adults and developed based on a review of the relevant literature and previous experience to ensure that it covered the key variables. However, it is important to note that the questionnaire has not been formally validated through methods such as reliability testing or validity assessment in the context of this study.

Socio-demographic questions gathered information on the participants' age, place of residence, education, and socio-economic status. Lifestyle questions included self-reported data on physical activity levels, alcohol consumption, smoking, use of dietary supplements, and the number of meals consumed per day. Parents assessed their physical activity levels by selecting one of five predefined categories, which ranged from very low, low, moderate, high, to very high. Each category included specific examples of exercise types and the corresponding weekly duration to guide respondents in making an appropriate selection [14]. Respondents were given a choice of three predefined frequencies of alcohol consumption, ranging from never, once a week or less, to 2–3 times/week. The questions on current use of dietary supplements (yes, no, which ones) included a short definition and gave some an example of them.

The group of children with food allergies and intolerances was identified based on parental declarations. Parents were specifically asked if their child's condition had been diagnosed by an allergist, the type of allergy and/or intolerance, and the child's age at the time of diagnosis.

Parents' nutritional knowledge was assessed using a questionnaire that included 30 questions in the following categories:

- general nutritional knowledge – included five basic questions about dietary recommendations, the number of meals consumed per day, the consumption of the main food groups such as vegetables and fruit, fish and seafood, meat and processed meats, and water intake;
- detailed nutritional knowledge – included five specific questions on the role of selected nutrients (protein, fat, carbohydrates, vitamin D, and iron), the effects of protein deficiency, and excessive consumption of simple sugars;
- nutritional knowledge of food allergies and intolerances – included twenty questions, (five questions in each subcategory) about identifying common allergens (questions about food products that are particularly common allergens), the occurrence of allergens in food products (questions about the most common sources of allergens in processed foods and their identification on product labels), an indication of alternative food products without allergens (questions to test knowledge of how to substitute products containing allergens), and dietary rules for eliminating gluten and lactose (including knowledge of the exclusion of products containing even traces of gluten or lactose).

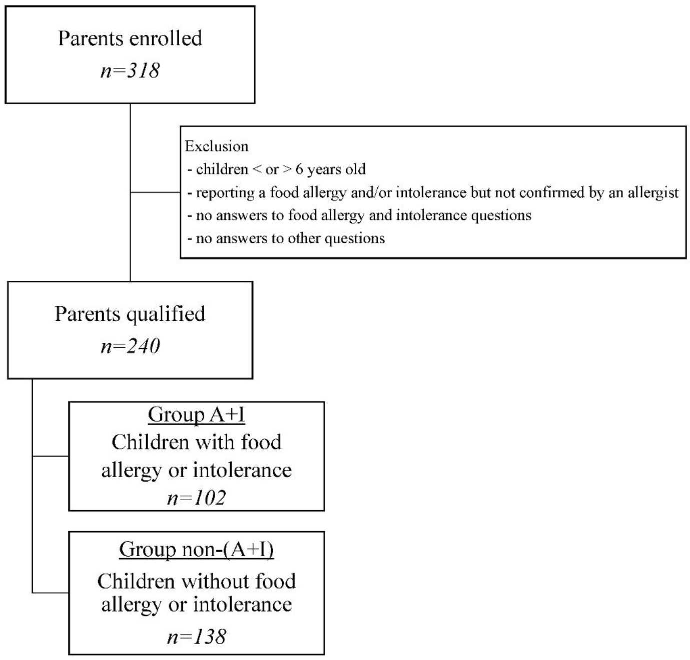


Figure 1. Study population flow chart

Source: own elaboration.

Each correct answer was worth 1 point and each incorrect answer was worth 0 points. Points were then added up within each category and subcategory. The maximum number of points that could be obtained in each category and subcategory was 5, so 5 points was considered very good, 4 good, 3 average, 2 poor, while 1 or less was considered very poor knowledge.

All survey procedures were conducted in accordance with the Declaration of Helsinki (1964) and its later amendments. The study was also approved by the Ethical Committee of the Warsaw University of Life Sciences (Resolution No. 25p/2018). The time required to complete the questionnaire was not burdensome and should not have exceeded 30 minutes. Apart from the information obtained from the questionnaire, no informed consent was required, as no other assignments were planned with the study participants.

Statistical analyses were performed using the Statistica software (version 13.4, StatSoft, USA). Characteristic data were presented as mean values ± standard deviation (*SD*) and as the percentage of parents. To determine differences in baseline characteristics between parents of children with a food allergy or intolerance and parents of children without these conditions, the Chi-square test was used for categorical variables. Based on the Shapiro-

Wilk test, the hypothesis of normality of the numerical variables (age and nutritional knowledge scores) was rejected; therefore, the Mann-Whitney U test was used to determine statistically significant differences between groups. *P*-values ≤0.05 were admitted as statistically significant.

Results and discussion

The mean age of both study groups was similar: parents of children with allergies and intolerances (A+I) had a mean age of 36.5 ±2.7 years, while parents of healthy children (non-(A+I)) had a mean age of 34.4 ±2.9 years (Tab. 1). Most parents lived in urban areas (82% in the allergic group and 98% in the non-allergic group). Both groups had similar levels of education, with higher education being the most common level observed (75% and 77%, respectively). A significant proportion of parents in both groups reported having a 'good' or 'very good' socio-economic status. Importantly, there were no statistically significant differences between the groups in these socio-demographic characteristics ($p > 0.05$).

Table 1. Characteristics of the studied group

Variables	Total (n = 240)	A+I (n = 102)	Non-(A+I) (n = 138)	*P*-value *
Age (years) mean ± *SD*	35.1 ±3.9	36.5 ±2.7	34.4 ±2.9	0.582
Residence (%)				
urban area	96	82	98	0.541
suburban area	4	18	2	
Education (%)				
primary	4	3	4	
secondary	14	18	11	0.221
vocational	6	4	8	
higher	76	75	77	
Socioeconomic status (%)				
average	17	18	17	0.699
good	41	42	40	
very good	42	40	43	
Physical activity (%)				
very low	0	0	0	
low	16	15	17	0.511
moderate	66	67	65	
high	18	18	18	
very high	0	0	0	
Drinking alcohol (%)				
never	43	45	42	0.443
once a week or less	48	47	49	
2–3 times/week	10	8	11	
Cigarette smoking (%)				
no	80	79	81	0.512
yes	20	21	19	
Dietary supplements use (%)				
no	57	54	59	0.336
yes	43	46	41	
Number of meals/day (%)				
≤3	23	22	24	0.389
4	40	40	40	
≥5	37	38	36	

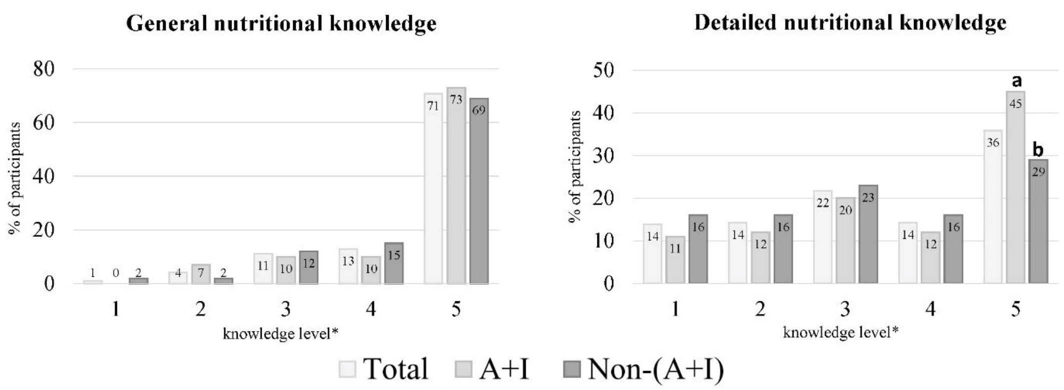
Group A+I – parents of children with food allergies or intolerances; non-(A+I) – parents of children without food allergies or intolerances. **P*-values were calculated using the Mann-Whitney's test for continuous variables and the chi-squared test for categorical data.

Source: own elaboration.

Physical activity levels were also similar in both groups, with most volunteers reporting moderate activity. Alcohol consumption was comparable, with the majority consuming alcohol 'once a week or less'. Smoking habits were nearly identical between the groups, and 43% of parents used dietary supplements. The largest group of participants (40%) reported eating four meals a day. There were no statistically significant differences in lifestyle factors between the groups ($p > 0.05$).

In group A+I, the most common allergies in children, as declared by the parents, included egg white (39%), peanuts (34%), fish and seafood (16%), and other allergens (11%), such as citrus fruits, tomatoes, strawberries, and soy. Lactose intolerance was the most common (74%), while gluten intolerance was demonstrated less frequently (26%). It should be emphasised that such a high proportion of individual allergies may be due to the limitations of this study. Allergies were not diagnosed directly and were based on parental report – only those questionnaires where parents confirmed the result obtained by a specialist were qualified. In addition, the sample selection for the study was based on the voluntary response sampling method, so it is possible that the subsequent lack of representativeness led to such a high proportion of individual food allergies and intolerances.

The majority of parents participating in the study (71%) demonstrated a very high level of general nutritional knowledge, regardless of whether their children had a food allergy or intolerance (73% and 69%, respectively); (Fig. 2). It is crucial for this group to have a high level of nutritional awareness, because they are responsible for shaping their children's dietary habits and behaviour. This increased awareness can be attributed to the many educational campaigns and programmes implemented in recent years, as well as the widespread availability of health and nutrition information [15]. Some researchers have observed a growing trend among adults to seek nutrition information from Internet sources and social media [16, 17]. While the Internet and social media are popular sources of nutrition information, it is important to note that they also carry the risk of disseminating inaccurate and unverified data, which often lacks scientific backing [18]. It is therefore important to encourage individuals to verify the information they receive from these sources, for example, by consulting a qualified dietitian. Some researchers have also found that despite the high levels of knowledge about basic nutrition principles, this knowledge does not always translate into healthier eating behaviours [19]. This phenomenon is particularly common among adults without medical conditions or health problems that require dietary restrictions [20]. A similar dependency has been observed in parents of children without food allergies or intolerances [21].



Group A+I – parents of children with food allergies or intolerances; non-(A+I) – parents of children without food allergies or intolerances; a, b – significant differences between the study groups, $p \leq 0.05$ (Mann-Whitney test); *scale of knowledge, where 1 indicates very poor, 2 poor, 3 average, 4 good and 5 points indicates very good knowledge.

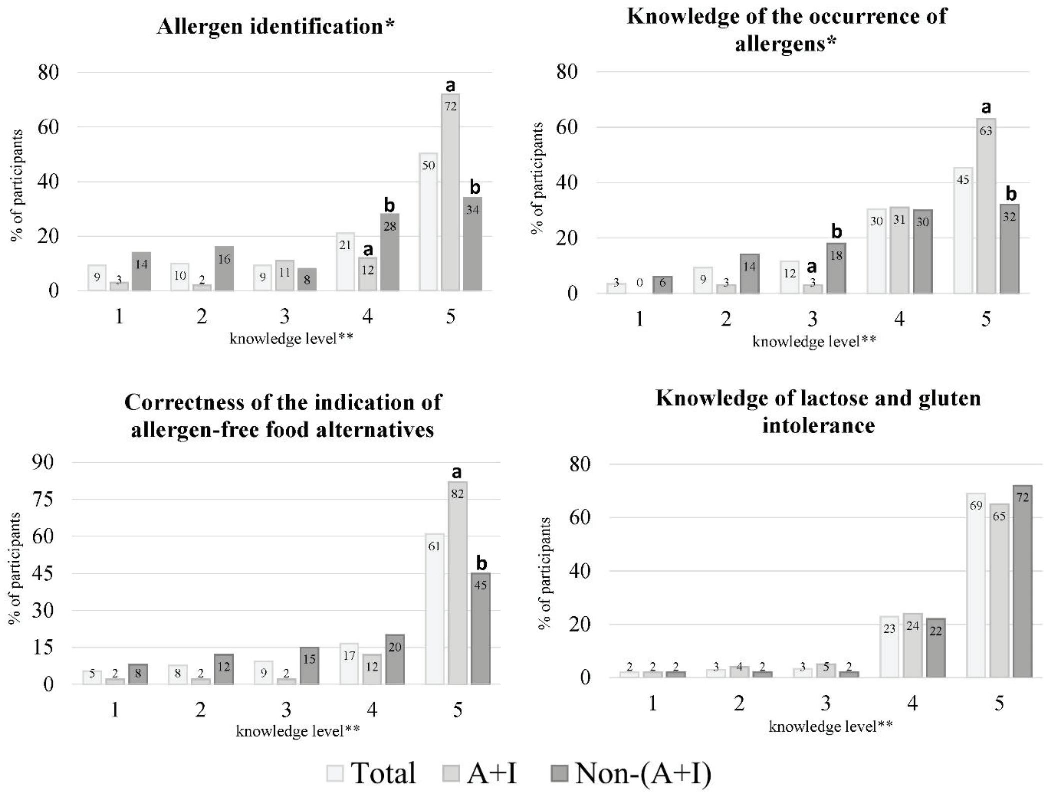
Figure 2. Results of the parents' general and detailed nutritional knowledge assessment

Source: own elaboration.

A significant proportion of participants had very good (36%) or good (14%) detailed nutritional knowledge. However, the A+I group had a statistically significantly higher level of very good knowledge (level 5) than the parents of healthy children (p = 0.005). Many participants in this group gave correct answers about the role of selected nutrients (protein, fat, carbohydrate, vitamin D, and iron) and the effects of protein deficiency and excessive intake of simple sugars. Several studies also confirm that people with food allergies were more motivated to learn about their diet [8, 22, 23]. They were significantly more likely to give correct information about the effects of specific nutrients on bodily functions, assess the consequences of nutrient deficiencies, and report that they frequently read labels and pay attention to the composition of the products they consume. It is, therefore, understandable that parents of healthy children were more likely to increase their knowledge about their children's nutrition.

In contrast, some of the parents studied had average or poor nutritional knowledge. Several studies have shown that people with food allergies and intolerances may be very knowledgeable about avoiding harmful substances, but only sometimes very knowledgeable about nutrition [24, 25]. In addition, people without allergies or intolerances often have misconceptions about the nutritional benefits of foods without problematic ingredients [26]. Many people without specific dietary needs mistakenly believe that allergen-free products are inherently healthier when these products may lack essential nutrients needed for proper body function [25].

The A+I group were statistically significantly more knowledgeable at the very good level (level 5) in identifying selected allergens compared to parents of healthy children ($p = 0.006$) (Fig. 3). In contrast, the non-(A+I) group was significantly more knowledgeable at the good level ($p = 0.003$). The vast majority of parents in the A+I group correctly identified allergens in ingredients such as shellfish (82% correct answers), fish (76%), eggs (78%), nuts (81%), soya (76%), as well as milk (85%) and hazelnuts (92%). An allergy or intolerance in a child often forces parents to increase their understanding of allergens. Studies by other authors support these results; people with food allergies or intolerances were more motivated to learn about allergen identification because of the direct health risks involved [27, 28, 29].



Group A+I – parents of children with food allergies or intolerances; non-(A+I) – parents of children without food allergies or intolerances. *Allergen identification (questions about food products that are particularly common allergens); occurrence of allergens in food products (questions about the most common sources of allergens in processed foods and their identification on product labels); a, b – significant differences between the study groups, $p \leq 0.05$ (Mann-Whitney test);**scale of knowledge, where 1 indicates very poor, 2 poor, 3 average, 4 good and 5 points indicates very good knowledge.

Figure 3. Results of the parents' nutritional knowledge of food allergies and intolerances assessment

Source: own elaboration.

At the same time, the A+I group had significantly better knowledge (at a very good level) than the parents of healthy children ($p = 0.002$) about the sources of allergens and their presence in processed foods. The vast majority of parents in the A+I group correctly identified the allergens on the labels of these foods (86% correct answers). A study by other researchers confirms that people with allergies tend to be vigilant and cautious in their food choices. They often showed better skills in reading labels and identifying potential allergens in food [30]. These individuals often become experts in identifying hidden allergens and understanding where potential cross-contamination may occur during food production or processing. This is crucial to avoid accidental exposure, especially when eating prepared meals in restaurants or canteens [7]. On the other hand, despite the increasing public awareness of allergens, many people still find it difficult to correctly identify hidden allergens, especially in processed foods [29, 31]. However, increasingly sophisticated allergen labelling systems are helping to make allergen identification much easier [9].

The studied parents of children with food allergies and intolerances (A+I group) had statistically significantly higher knowledge at a very good level (level 5) in identifying allergen-free alternatives than the non-(A+I) group (p = 0.004). Most parents of sick children correctly indicated that the substitutes must be completely free of allergens (78%). They were also able to identify specific equivalents of products, replacing products containing allergens correctly (average 86% correct answers). People with food allergies or intolerances often have greater knowledge of such products because they actively seek safe substitutes for foods they cannot consume. This includes familiarity with specific brands and products designed for allergen-sensitive consumers [11, 32]. Developing specialised markets, such as gluten- or lactose-free products, has significantly improved the availability and labelling, making it easier for people with allergies to identify foods that are safe for them [31]. However, some researchers suggest that despite the increase in allergen-free products, their marketing may mislead healthy individuals into believing that these products are healthier or necessary for everyone. This misconception may lead to unnecessary dietary changes for those without allergies [6].

The awareness of lactose and gluten intolerance was similar in both groups and again the highest proportion of participants had a very good level of knowledge (level 5). There has been a significant increase in public awareness of these intolerances, linked to the increasing availability of gluten- and lactose-free products and the increased presence of these issues in the media. Social media and marketing by manufacturers of these types of foods play an important role [33, 34]. However, it is important to note that this awareness does not always translate into an understanding of the medical necessity of such diets [10, 35]. Despite the high level of awareness, many people follow gluten-free or lactose-free diets because of the perceived health benefits rather than the actual need due to intolerance to these ingredients. This trend towards unjustified dietary restrictions can lead to serious nutrient deficiencies [12].

The strength of the study was the participation of many parents, both parents of children with food allergies or intolerances and parents of healthy children. The clear comparison between the two groups provided detailed insight into how allergies and intolerances affect parents' knowledge, particularly in identifying allergens and product alternatives free of problematic ingredients. However, the study had several limitations. It was based on a voluntary response sampling method, which prevented the collection of a representative sample. It is important to note that, although the questionnaire was carefully designed to collect the necessary data, it was not formally validated. In addition, information on the presence of food allergies and intolerances was obtained only on the basis of the parents' declaration of a medical diagnosis by a specialist, which may introduce a degree of reporting bias. Another limitation of the study was the need to reduce the number of questions assessing the knowledge about nutrition and food allergies and intolerances, as the longer questionnaire was met with a negative response and a low number of completed responses. This limited the ability to use more advanced statistical methods.

CONCLUSIONS AND FUTURE PERSPECTIVES

In summary, the results indicate that most parents, regardless of their children's health status, had a high level of general nutrition knowledge. Parents of children with food allergies or intolerances showed significantly higher detailed knowledge, particularly in areas such as allergen identification and better identification of allergen-free food alternatives. These results highlight the need for targeted education to improve nutritional knowledge, especially among parents of children without food allergies or intolerances. A better understanding of the more specific issues related to nutrition can have a significant impact on the proper nutrition and development of their children.

Acknowledgement
The research was financed by the Polish Ministry of Science and Higher Education with funds of the Institute of Human Nutrition Sciences.

REFERENCES

[1] **Alves J.G.B, Alves L.V. 2024.** Early-life nutrition and adult-life outcomes. The Journal of Pediatrics 100(1): S4–S9. https://doi.org/10.1016/j.jped.2023.08.007

[2] **Roberts M., Tolar-Peterson T., Reynolds A., Wall C., Reeder N., Rico Mendez G. 2022.** The Effects of Nutritional Interventions on the Cognitive Development of Preschool-Age Children: A Systematic Review. Nutrients 14, 532: 1-15. https://doi.org/10.3390/nu14030532

[3] **Elghoudi A., Narchi H. 2022.** Food allergy in children-the current status and the way forward. World Journal of Clinical Pediatrics 11: 253–269. https://doi.org/10.5409/wjcp.v11.i3.253
[4] **Wright K., Feeney M., Yerlett N., Meyer R. 2022.** Nutritional Management of Children with Food Allergies. Current Treatment Options in Allergy 9: 375–393. https://doi.org/10.1007/s40521-022-00320-7
[5] **Turner P.J., Campbell D.E., Boyle R.J., Levin M.E. 2018.** Primary Prevention of Food Allergy: Translating Evidence from Clinical Trials to Population-Based Recommendations. The Journal of Allergy and Clinical Immunology: In Practice 6: 367–375. https://doi.org/10.1016/j.jaip.2017.12.015
[6] **Chafei H., El Harake M. D., Toufeili I., Kharroubi S. A. 2023.** Knowledge, Attitudes, and Practices of Consumers on Food Allergy and Food Allergen Labeling: A Case of Lebanon. Foods (Basel, Switzerland) 12(5), 933: 1–15. https://doi.org/10.3390/foods12050933
[7] **Zurzolo G.A., Mathai M.L., Koplin J.J., Allen K.J. 2012.** Hidden allergens in foods and implications for labelling and clinical care of food allergic patients. Current Allergy and Asthma Reports 12 :292–296. https://doi.org/10.1007/s11882-012-0263-6
[8] **Soos G., Lugasi A. 2024.** Consumer attitude research regarding food hypersensitivity. International Journal of Gastronomy and Food Science 36, 100918: 1–11. https://doi.org/10.1016/j.ijgfs.2024.100918
[9] **Dinardo G., Fierro V., Del Giudice M.M., Urbani S., Fiocchi A. 2023.** Food-labeling issues for severe food-allergic consumers. Current Opinion in Allergy and Clinical Immunology 23: 233–238. https://doi.org/10.1097/ACI.0000000000000902
[10] **Zerbini C., De Canio F., Martinelli E., Luceri B. 2024.** Are gluten-free products healthy for non-celiac consumers? How the perception of well-being moderates gluten-free addiction. Food Qual Prefer 118, 105183: 1–11. https://doi.org/10.1016/j.foodqual.2024.105183
[11] **Smits N.G.E., Pastorelli A.A., Ballin N.Z., Bruggeman C., Poucke C., Voorhuijzen-Harink M.M, Westphal Y., Walker M., Winkel M., Koops A.J. 2025.** The changing food allergen landscape in Europe calls for harmonised food allergen monitoring: Position paper. Food Control 168, 110915: 1–9. https://doi.org/10.1016/j.foodcont.2024.110915
[12] **Rubio-Tapia A., Hill I. D., Semrad C., Kelly C. P., Greer K. B., Limketkai B. N., Lebwohl B. 2023.** American College of Gastroenterology Guidelines Update: Diagnosis and Management of Celiac Disease. The American journal of gastroenterology 118(1), 59–76. https://doi.org/10.14309/ajg.0000000000002075
[13] **Parasuraman S.R., Ghandour R.M., Kogan M.D. 2020.** Epidemiological Profile of Health and Behaviors in Middle Childhood. Pediatrics 145, e20192244: 1–12. https://doi.org/10.1542/peds.2019-2244
[14] **Bull F.C., Al-Ansari S.S., Biddle S. et al. 2020.** World Health Organization 2020 guidelines on physical activity and sedentary behaviour. British Journal of Sports Medicine 54: 1451–1462.
[15] **Doustmohammadian A., Bazhan M. 2021.** Social marketing-based interventions to promote healthy nutrition behaviors: A systematic review protocol. Systematic Reviews 10, 75: 1–8. https://doi.org/10.1186/s13643-021-01625-5
[16] **Spronk I., Kullen C., Burdon C., O'Connor H. 2014.** Relationship between nutrition knowledge and dietary intake. British Journal of Nutrition 111: 1713–1726. https://doi.org/10.1017/S0007114514000087
[17] **Carbonneau E., Lamarche B., Provencher V. et al. 2021.** Associations Between Nutrition Knowledge and Overall Diet Quality: The Moderating Role of Sociodemographic Characteristics – Results from the PREDISE Study. American Journal of Health Promotion 35: 38–47.
[18] **Kliemann N., Wardle J., Johnson F., Croker H. 2016.** Reliability and validity of a revised version of the General Nutrition Knowledge Questionnaire. European journal of clinical nutrition 70(10): 1174–1180. https://doi.org/10.1038/ejcn.2016.87
[19] **Scalvedi M.L., Gennaro L., Saba A., Rossi L. 2021.** Relationship between nutrition knowledge and dietary intake: An assessment among a sample of Italian adults. Frontiers in Nutrition 8, 714493: 1–13. https://doi.org/10.3389/fnut.2021.714493
[20] **Spronk I., Kullen C., Burdon C., O'Connor H. 2014.** Relationship between nutrition knowledge and dietary intake. British Journal of Nutrition 111: 1713–1726. https://doi.org/10.1017/S0007114514000087
[21] **Kraus A., Annunziata A., Vecchio R. 2017.** Sociodemographic Factors Differentiating the Consumer and the Motivations for Functional Food Consumption. Journal of the American College of Nutrition 36(2), 116–126. https://doi.org/10.1080/07315724.2016.1228489
[22] **Valero-Moreno S., Torres-Llanos R., Pérez-Marín M. 2024.** Impact of Childhood Food Allergy on Quality of Life: A Systematic Review. Applied Sciences, 14(23), 10989: 1–18. https://doi.org/10.3390/app142310989
[23] **Saadati K., Kordbageri M.R., Chaboksavar F., Jahangasht Ghoozlu K., Parvizi S., Shamsalinia A., Ghadimi R., Porasgari Z., Ghaffari F. 2024.** Investigating the mediating role of food involvement in the relationship between eating restrictions, nutritional knowledge, and dietary patterns in adults. PLoS One 19, e0301533: 1–19. https://doi.org/10.1371/journal.pone.0301533
[24] **Ares G., Giménez A., Gámbaro A. 2008.** Consumer perceived healthiness and willingness to try functional foods. Appetite 79: 34–41. https://doi.org/10.1016/j.appet.2008.05.061
[25] **Anagnostou A. 2021.** Addressing Common Misconceptions in Food Allergy: A Review. Children 8, 497: 1–13. https://doi.org/10.3390/children8060497

[26] **Gupta R.S., Springston E.E., Kim J.S., Smith B., Pongracic J.A., Wang X., Holl J. 2010.** Food allergy knowledge, attitudes, and beliefs of primary care physicians. Pediatrics 125: 126–132. https://doi.org/10.1542/peds.2009-1116

[27] **Cornelisse-Vermaat J.R., Voordouw J., Yiakoumaki V., Theodoridis G., Frewer L.J. 2008.** Food-allergic consumers' labelling preferences: A cross-cultural comparison. European Journal of Public Health 18: 115–120. https://doi.org/10.1093/eurpub/ckm032

[28] **Voordouw J., Cornelisse-Vermaat J.R., Yiakoumaki V., Theodoridis G., Chryssochoidis G., Frewer L.J. 2009.** Food allergic consumers' preferences for labelling practices: a qualitative study in a real shopping environment. International Journal of Consumer Studies 35: 348–355. https://doi.org/10.1111/j.1470-6431.2008.00735.x

[29] **Marra C. A., Harvard S., Grubisic M., Galo J., Clarke A., Elliott S., Lynd L. D. 2017.** Consumer preferences for food allergen labeling. Allergy, Asthma and Clinical Immunology 13, 19: 1–11. https://doi.org/10.1186/s13223-017-0189-6

[30] **Reese I., Holzhauser T., Schnadt S., Dölle S., Kleine-Tebbe J., Raithel M., Worm M., Zuberbier T., Vieths S. 2015.** Allergen and allergy risk assessment, allergen management, and gaps in the European Food Information Regulation (FIR): Are allergic consumers adequately protected by current statutory food safety and labelling regulations?. Allergo journal international 24: 180–184. https://doi.org/10.1007/s40629-015-0066-0

[31] **Barnett J., Begen F.M., Gowland M.H., Lucas J.S. 2018.** Comparing the eating out experiences of consumers seeking to avoid different food allergens. BMC public health, 18(1), 1263: 1–12. https://doi.org/10.1186/s12889-018-6117-y

[32] **Turner P.J., Mehr S., Joshi P., Tan J., Wong M., Kakakios A., Campbell D.E. 2015.** Safety of food challenges to baked egg or baked milk: A systematic review and meta-analysis. Pediatric Allergy and Immunology 26: 547–555. https://doi.org/10.1111/pai.12093

[33] **Bryła P., Chatterjee S., Ciabiada-Bryła B. 2022.** Wpływ marketingu w mediach społecznościowych na zaangażowanie konsumentów w zrównoważoną konsumpcję: systematyczny przegląd literatury. International Journal of Environmental Research and Public Health 19, 16637: 1–16. https://doi.org/10.3390/ijerph192416637

[34] **El Khoury D., Balfour-Ducharme S., Joye I.J. 2018.** A review on the gluten-free diet: Technological and nutritional challenges. Nutrients 10, 1410: 1–25. https://doi.org/10.3390/nu10101410

[35] **Aljada B., Zohni A., El-Matary W. 2021.** The Gluten-Free Diet for Celiac Disease and Beyond. Nutrients 13, 3993: 1–22. https://doi.org/10.3390/nu13113993

DOI: 10.22630/TPFP.2024.1.10071

ORIGINAL PAPER
Received: 22.11.2024
Accepted: 27.01.2025

ANALYSIS OF THE PHYSICOCHEMICAL PROPERTIES OF MILK THISTLE SEED OIL AND EVALUATION OF ITS BIOACTIVE POTENTIAL

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Abstract: Milk thistle (*Silybum marianum* L. Gaertn.) is the Asteraceae family's extensively distributed herbaceous plant. Its seeds are famous for their medicinal and industrial potential due to their lipid fraction, richness in valuable fatty acids, and bioactive compounds, including silymarin and polyphenolics. The study aimed to evaluate the chemical composition and antioxidant properties of commercial milk thistle seed oil. The fatty acid profile, triacylglycerol distribution, thermal property, and antioxidant activity were analysed. Research on the physicochemical properties of Polish cold-pressed oil from milk thistle seeds has shown that this oil is a valuable source of unsaturated fatty acids, mainly linoleic (49.83 ±0.02%) and oleic (27.36 ±0.11%). Beneficial nutritional and health values characterise it, but due to the high value acid number (4.43 ±0.14 mg KOH/g) and low polyphenol content (0.559 ±0.004 mg GAE/g), it is unstable, quickly turns rancid, and loses its sensory properties.

Key words: milk thistle seed oil, fatty acids profile, antioxidant properties, oxidative stability

INTRODUCTION

In recent years, oils derived from non-conventional seeds have gained increasing significance. These include oils extracted from, among others, flaxseeds, safflower, pumpkin seeds, grape seeds, pomegranate seeds, and milk thistle seeds [1]. The popularity of oils from unconventional oil seeds, especially those rich in health-promoting ingredients [2], results from the increasing awareness of consumers, their care for their health, and disease prevention through the proper selection and balance of dietary ingredients. The principles of healthy eating are associated with limiting the consumption of saturated fatty acids, which is why there is growing interest in alternative plant oils characterised by a high content of unsaturated fatty acids.

Milk thistle (*Silybum marianum* L. Gaertn.), a member of the Asteraceae family, is a herbaceous plant native to the Mediterranean Basin, North Africa, and the Middle East [3–5], however, the plant is presently widespread throughout the world. Poland is a significant European producer of milk thistle seeds. Currently, the domestic variety, Silma, is cultivated on plantations and is characterised by a high yield of achenes (approx. 1.5 t/h) [6].

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Milk thistle is commercially cultivated for its remarkable medicinal properties [7]. It is a safe and well-tolerated herbal product with no known health risks or side effects. The leading pharmaceutical compound of milk thistle is a mixture of at least six flavonolignans, popularly called silymarin. It consists mainly of silibinin, isosilibinin, silychristin, isosilychristin, silydianin, and one flavonoid – taxifolin. Silibinin is the predominant compound in silymarin and is considered to be the primary active ingredient [7–9]. According to Zheljazkov et al. [10], the contents of individual active ingredients in milk thistle seeds are at the level of 0.26–0.36% for taxifolin, 0.69–0.99% for silydianin, 0.27–0.39% for isosilibinin and 1.31–1.78% for silibinin, respectively. In addition to the flavonoid complex, milk thistle seeds contain quercetin, histamine, tyramine, phytosterols, tannins, organic acids, and mineral compounds [11,12]. They also contain high concentrations of oil (20–30%), protein (25–30%) and sugars (approx. 37%) [13–15].

Since the oil must be removed from the seeds before the silymarin can be extracted, milk thistle seed oil is an essential by-product worthy of attention. It has a high concentration of unsaturated fatty acids, which are beneficial to human health in preventing arteriosclerosis, diabetes, and cancer [16]. Moreover, milk thistle seed oil is recommended as a potential source of a natural antioxidant – vitamin E [17]. It also contains other valuable ingredients, including micro and macro elements, at a significant level, such as calcium, potassium, and copper (17 mg/g) [1,18]. In China, since 2014, the Chinese Ministry of Health has approved milk thistle seed oil as a new source of edible oil, allowing its use except for infant food [19].

In recent years, many studies have been conducted to better demonstrate the benefits of milk thistle seed oil. In Poland, milk thistle seeds and the oil that can be extracted from them have been becoming increasingly popular. This research was aimed at assessing the physicochemical and health-promoting properties of *Silybum marianum* L. seed oil extracted by cold pressing (at a temperature not exceeding 40°C), unrefined. This work shows the fatty acid profile, triacylglycerol distribution, thermal property, antioxidant activity and health indicators of milk thistle seed oil. These results contribute to a comprehensive understanding of the role of milk thistle seed oil and complement the existing knowledge of its physicochemical properties.

MATERIAL AND METHODS

Materials

The research material was milk thistle seed oil. It came from the commercial offer of Białystok PPHU JS Słodkie Zdrowie company and Szamotuły SemCo Sp. z o.o. (Poland).

It was cold pressed, unrefined oil. The oil was tested in the first month after pressing, within its declared expiration date. The oil was stored in a refrigerator at approximately 10°C during the analysis. All reagents and solvents, including hexane, ethanol, methanol, diethyl ether, potassium hydroxide, sodium carbonate, acetic acid, and chloroform, were purchased from Sigma-Aldrich and were of analytical quality. The solvents used for the spectrophotometric and chromatographic measurements were of HPLC grade

Determination of fatty acid composition

The compositions of fatty acids in the commercial milk thistle oils were determined using gas chromatography (GC), with a capillary column and a flame ionisation detector. The EN ISO 5509:2001 standard [20] was applied to determine the composition of fatty acids present in the oil. To generate volatile derivatives of fatty acids, the studied samples of oil were applied to esterification with methanol, resulting in fatty acid methyl esters (FAME). A gas chromatograph (YL6100 GC), equipped with a BPX-70 capillary column (0.25 µm film thickness, 60 m length, and 0.25 mm internal diameter), was used to analyse the samples. The carrier gas inside the column was nitrogen. The FAME separation conditions were as follows: initial temperature of 70°C was maintained for 0.5 min, the temperature rise increment was 15°C/min within the range from 70°C to 160°C, then the temperature rise increment was 1.1°C/ min within the range from 160°C to 200°C, and finally 30°C/min within the range from 200°C to 225°C. The end temperature of 225°C was maintained for 15 min; the temperatures of the detector and injector were 250°C and 225°C, respectively. Fatty acids were identified based on retention time values compared with standards purchased from Sigma Aldrich, Supelco Analytical, Bellefonte, PA, USA [21].

Determination of triacylglycerol structure using enzymatic hydrolysis

The distributions of fatty acids in commercial milk thistle oils were determined with regard to their positions – internal or external of triacylglycerols – in accordance with the methods described in papers by Bryś et al. [21]. The analysed oils were subjected to enzymatic hydrolysis using pancreatic lipase, which breaks the ester bonds in the external positions of triacylglycerols. The resulting products were extracted with diethyl ether. Following that, the products of enzymatic deacylation of triacylglycerols, dissolved in ether, were separated using preparative thin-layer chromatography. Isolated *sn*-2 monoacylglycerols, together with a gel, were removed from chromatoplates, followed by their elution with diethyl ether. The fatty acid composition of the obtained *sn*-2 monoacylglycerols was determined by gas chromatography. Based on the compositions of isolated *sn*-2 monoacylglycerols and the starting triacylglycerols, the composition of the fatty acid in the *sn*-1,3 positions was determined.

Determination of acid value and free fatty acid content

The degree of hydrolysis of the analysed oils was determined by the acid value according to the ISO 660:2009 method [22]. The acid value was determined using titration of oil samples dissolved in the mixture of diethyl ether:ethanol (1:1, v/v) with 0.1 M ethanolic potassium hydroxide solution. The free fatty acids content was then computed using the value of the molar mass of oleic acid and the acid value for the studied samples.

Determination of peroxide value

The content of the primary oxidation products of the oils was examined by the peroxide value according to the ISO 3960:2007 method [23]. The peroxide value was determined by the iodometric technique.

Determination of oxidation induction time

Pressure Differential Scanning Calorimetry (PDSC) was used to define the oxidative stability of milk thistle oil. To determine the induction time for the oxidation reaction of the oils, experiments were carried out with the help of a DSC Q20 apparatus (TA Instruments) linked to a high-pressure chamber. The weight of the oil used in the test ranged from 3 to 4 mg. Oil samples were placed in small aluminium pans in an oxygen atmosphere under a pressure of 1,400 kPa. Measurements were taken isothermally at 120°C. The oxidation induction time was determined from the PDSC curves.

Health indicators of oil

The composition of fatty acids was used to calculate health indicators. The hypocholesterolemia/hypercholesterolemia (h/H) ratio was obtained using Eq. 1, and the atherogenicity index (AI) and thrombogenicity index (TI) were obtained using Eq. 2 and 3, respectively.

$$h/H = \frac{\left(\text{cis-C18:1} + \sum \text{PUFA}\right)}{\left(\text{C12:0} + \text{C14:0} + \text{C16:0}\right)} \tag{1}$$

$$AI = \frac{\left(\text{C12:0} + \left(4 \times \text{C14:0}\right) + \text{C16:0}\right)}{\left(\sum PUFA\right)} \tag{2}$$

$$TI = \frac{\left(\text{C14:0} + \text{C16:0} + \text{C18:0}\right)}{(0.5 \times \sum \text{MUFA} + \left(0.5 \times \sum \text{n-6PUFA}\right) + \left(3 \times \sum \text{n-3PUFA}\right) + \frac{\left(\text{n-3}\right)}{\left(\text{n-6}\right)}} \tag{3}$$

Determination of total phenolic content

The phenolic content in the samples was quantified using Folin-Ciocalteu's reagent. One gram of each *Silybum marianum* L. seed oil was dissolved in 5 ml of *n*-hexane and extracted with 5 ml of methanol by vortexing at ambient temperature. After centrifugation, the methanolic layer was separated from the lipid phase. Subsequently, 0.5 ml of the methanolic layer was diluted in water, and then 0.5 ml of Folin-Ciocalteu's reagent and 1 ml of a sodium carbonate solution (20%) were added. The absorbance was measured at 760 nm after 60 minutes with the samples kept in the dark. The total phenolic content in the sample was determined using a standard curve plotted for gallic acid ($y = 8086x + 0.0237$; $R^2 = 0.9952$). The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of oil.

DPPH scavenging activity

The antioxidant power was estimated by the DPPH (2,2′-diphenyl-1-picrylhydrazyl) test. The DPPH solution was prepared by dissolving 10 mg of DPPH in 100 mL of methanol. 50 µL of the methanolic solutions of the oil sample was added to 2.95 mL of the methanolic solution of DPPH. The mixture was shaken vigorously and left at room temperature in the dark for 30 minutes. Then the absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (RAYLLEIGH UV-1601). The inhibition of the free radical DPPH as a percentage [I(%)] was calculated as follows (Eq. 4):

$$Inh\ (\%) = 100 \times \frac{\left(A_{\text{blank}} - A_{\text{sample}}\right)}{A_{\text{blank}}}, \tag{4}$$

where:
A_{blank} – the absorbance of the control (containing all reagents except the test compound),
A_{sample} – the absorbance of the test compound.

Data analysis

All the analyses were performed in triplicate and the results were expressed as mean values ±standard deviations (*SD*). The statistical analysis was performed using the Statistica software, version 13.3 (StatSoft, Krakow, Poland). Analysis of variance (ANOVA) was used. A *p*-value of ≤0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Fatty acid profile in milk thistle seeds oil

The composition of fatty acids is a crucial characteristic of edible oil, which has a huge influence on its physicochemical and nutritional properties. Their composition in the tested cold-pressed milk thistle seed oils is shown in Table 1. In the oil samples studied, 11 types of fatty acids were identified based on GC analysis. Linoleic (C18:2, 49.83 ±0.02%; 48.97 ±1.02), oleic (C18:1, 27.36 ±0.11%; 27.06 ±0.09), palmitic (C16:0, 8.47 ±0.02%; 10.39 ±1.35) and stearic (C18:0, 6.23 ±0.11%; 5.33 ±0.02) were the most predominant fatty acids, which accounted for more than 90% of the total fatty acids. Polyunsaturated acids ware present in more than twice the concentration of monounsaturated acids, and the ratio of unsaturated to saturated was approx. 4.31 ±0.22 and 3.95 ±0.02, respectively, for Białystok PPHU JS Słodkie Zdrowie and Szamotuły SemCo Sp. z o.o. company.

Table 1. Fatty acid composition of commercial milk thistle seeds oils

Fatty acid	Fatty acids composition [%]	
	thistle seeds oils from Białystok PPHU JS Słodkie Zdrowie company	thistle seeds oils from Szamotuły SemCo Sp. z o.o. company
C14:1	0.10 ±0.01^{a}	–
C16:0	8.47 ±0.02^{b}	10.39 ±1.35^{b}
C16:1	0.06 ±0.01^{a}	0.04 ±0.01^{a}
C18:0	6.23 ±0.11^{b}	5.33 ±0.02^{b}
C18:1 *n*-9	27.36 ±0.11^{c}	27.06 ±0.09^{c}
C18:2 *n*-6	49.83 ±0.02^{d}	48.97 ±1.02^{d}
C18:3 *n*-3	0.20 ±0.01^{a}	0.11 ±0.07^{a}
C20:0	3.76 ±0.03^{a}	4.28 ±0.12^{b}
C20:1	1.11 ±0.02^{a}	2.88 ±0.08^{a}
C20:4 *n*-6	2.51 ±0.17^{a}	1.21 ±0.12^{a}
C24:0	0.40 ±0.04^{a}	0.32 ±0.08^{a}
SFA	18.85 ±0.05	20.32 ±0.03
MUFA	28.62 ±0.03	29.98 ±0.10
PUFA	52.54 ±0.10	50.29 ±0.09
U/S	4.31 ±0.22	3.95 ±0.02

Note: Results presented in the table are the mean ± standard deviation ($n = 3$). Values in the same row with different letters are statistically significantly different ($p < 0.05$). SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; U/S – unsaturated fatty acid/saturated fatty acid.

Source: own elaboration.

The results for the percentage of fatty acid composition of milk thistle seed oil in this study differ slightly from published data. Meddeb et al. [4] analysed the fatty acid composition of oil from three varieties of milk thistle seeds from different areas in Tunisia (Bizerte, Zaghouan, and Sousse). Among the fatty acids, linoleic acid was the most abundant, accounting for 57.0% to 60.3%. The content of oleic acid was 15.5–22.4% and palmitic acid was 5.5–11.4%. Hassanein et al. [24] reported 53.3% for linoleic, 20.8% for oleic, 9.4% for palmitic, and 6.6% for stearic acid in the milk thistle seed oil native to Egypt. In addition, Fathi-Achachlouei and Azadmard-Damirchi [3] observed 49.7–53.6% for linoleic, 22.8–28.9% for oleic, 7.3–8.4% for palmitic, and 4.6–6.8% for stearic acid present in Iranian milk thistle seed oil. The differences indicate that the fatty acid composition of milk thistle seed oil depends on the geographical location and genotype. The presence of more linoleic and less oleic acid in milk thistle seed oil makes it valuable in terms of its nutritional value, but more susceptible to oxidation processes compared to the popular rapeseed or sunflower oil.

Physicochemical characterisation of cold pressed milk thistle seed oil

In order to more fully characterise the milk thistle seed oil, further research determined the acid and peroxide values, oxidation induction times (Table 2) and the composition of fatty acids in the external (*sn*-1,3) and internal (*sn*-2) positions of triacylglycerols (Table 4).

The acid value is an indicator of the degree of fat hydrolysis. It is defined as milligrams of KOH needed to neutralise the free fatty acids present in 1 g of oil. According to the Codex Alimentarius, the acid value in edible oil should not be higher than 4 mg KOH/g of oil. The conducted research shows (Table 2) that Polish commercial milk thistle oil is characterised by a quite high acid value – 4.43 ±0.14 mg KOH/g and thus exceeds the permissible normative value. An increased acid value is associated with an increased content of free fatty acids. The oil contained approximately 2.23% of free fatty acids, which were formed as a result of enzymatic hydrolysis by lipases, which could have occurred during the storage of seeds and oil. Therefore, it is recommended that seeds should be processed without long storage times, and seeds and oil should be stored at low temperature and low humidity [25]. Additionally, it is worth noting that FFAs are more susceptible to oxidation than the fatty acids that are present in the triacylglycerol molecules. Hence, the higher the level of FFAs in the seed oil, the higher the risk of oxidation. This phenomenon causes the oil to become rancid, which negatively affects its sensory value.

Table 2. Acid value (AV), free fatty acid (FFA) content, peroxide value (PV) and oxidation induction time (OIT) of analysed oils

Specification	AV [mg KOH/g]	FFA [%]	PV [meq O_2 /kg]	OIT [min]
Thistle seeds oil from Białystok PPHU JS Słodkie Zdrowie	4.43 ±0.14	2.23 ±0.07	24.16 ±4.22	31.54 ±1.99
Thistle seeds oil from Szamotuły SemCo Sp. z o.o.	6.02 ±0.11	3.45 ±0.23	22.11 ±3.02	28.72 ±0.13

Source: own elaboration.

An indicator of the content of primary oxidation products is the peroxide value. It is defined as the number of ml of standard sodium thiosulphate solution needed to titrate the iodine separated from a potassium iodide solution as a result of the action of the peroxides contained in 1 g of oil. Peroxide value is expressed in milliequivalents of oxygen per kg of oil. The peroxide value for cold-pressed oils in accordance with the Codex Alimentarius should not exceed 15 meq O_2/kg and, according to the PN-EN ISO 3960:2017-03 [26] standard – 10 meq O_2/kg. Research on the peroxide value in the tested oils (Table 2 – PV over 24 meq O_2/kg oil) showed that the content of primary oxidation products significantly exceeded both the Codex Alimentarius standard and the ISO standard. The peroxide value of the tested oil differs significantly from the value of this parameter determined in milk thistle oils from other parts of the world. The acid and peroxide values of the various milk thistle seed oils characterised by other scientists were very low, even lower than those determined by the Codex Alimentarius [4, 27,28]. Comparing the results obtained in this study with the results of other researchers examining freshly pressed oil, it can be concluded that the oils immediately after pressing were characterised by a lower content of peroxides than commercial oils [4, 27]. The higher acid and peroxide values of the tested milk thistle seed oil indicate that this type of oil, cold pressed, without refining should be consumed in a short time. The acid and peroxide values may also have been exceeded due to poor storage of the oil or poor quality of the seeds intended for pressing, as well as an incorrect pressing process itself.

In order to provide a more complete characterisation of the analysed oil, samples were also tested using Pressure Differential Scanning Calorimetry (PDSC). The PDSC tests conducted at an isothermal temperature of 120°C showed an average 30.13-minute oxidation induction time for the tested oil (Figure 1).

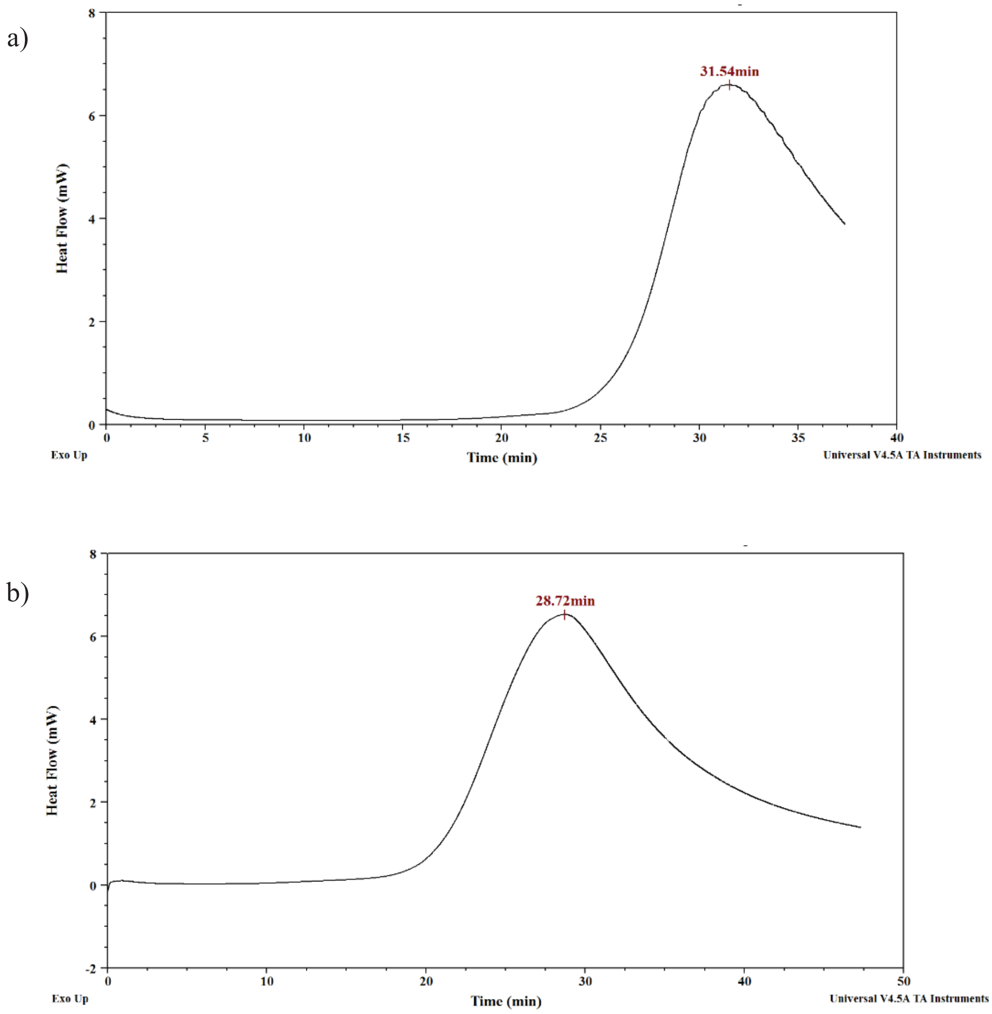

Figure 1. PDSC curves of commercial milk thistle seeds oil where: a) – PDSC curve for thistle seeds oils from Białystok PPHU JS Słodkie Zdrowie; b) – Thistle seeds oils from Szamotuły SemCo Sp. z o.o.

Source: own elaboration.

This value was slightly lower compared to milk thistle oil (about 33 min), which was the subject of research by Bryś et al. [21] on a human milk fat substitute obtained from mixtures of lard and milk thistle oil. Scientists also tested lard using the same method, for which the induction time was over 45 minutes. In general, oils contain a high concentration of unsaturated fatty acids, which are characterised by short induction times and, consequently, worse oxidative stability. Therefore, special attention should be paid to oils such as milk thistle oil during processing and storage, as they are very susceptible to oxidative changes and partial loss of quality.

Analysis of health indices for tested commercial milk thistle seeds oil

Specific health indicators of hypocholesterolemia/hypercholesterolemia, atherogenicity and thrombogenicity provide information on the impact of the fatty acids present in oils on human health in terms of the risk of atherosclerosis and the likelihood of thrombosis. The oils recommended for consumption are characterised by a high hypocholesterolemia/hypercholesterolemia (h/H) index and low atherogenicity (AI < 1.0) and thrombogenicity (TI < 0.5) indices. Consumption of products with a lower AI is associated with a reduction in total cholesterol levels and low-density lipoprotein (LDL) cholesterol in human plasma, while consuming products with a lower TI and a higher h/H ratio may be beneficial in the prevention of cardiovascular heart disease [29]. The analysed oil was characterised by a low atherogenicity index (AI = 0.16) and a bit high thrombogenicity index (TI = 0.90); (Table 3). Milk thistle oil, which is the subject of this research, is also characterised by the highest h/H index value (9.44). Summarising the obtained results, it can be stated that the tested oil is characterised by very beneficial nutritional and health values.

Table 3. Health indices for tested commercial milk thistle seeds oil

Health indices		
AI*	TI	h/H
0.161	0.899	9.438

Note: AI – atherogenic index; TI – thrombogenic index; h/H – hypocholesterolaemic/hypercholesteraemic index.

Source: own elaboration.

Analysis of fatty acid composition in external and internal positions of triacylglycerols

Based on enzymatic hydrolysis and chromatographic analysis of the products, the structure of the triacylglycerols in the tested oil was also examined. Taking into account the obtained results (Table 4, Figure 2), it can be concluded that in the tested oil, saturated fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0) are found mainly in the external positions of triacylglycerols, because their share in the central TAG position is very small. However, unsaturated acids such as oleic (C18:1) and linoleic (C18:2) acids occupy mainly the internal TAG position, as their share in this position is over 80% (Figure 2).

The structure of triacylglycerols is responsible, among other things, for the proper absorption of fats and oil from food. It also prevents the formation of insoluble calcium salts. This is because pancreatic lipase is an enzyme that selectively hydrolyses fatty acids in the sn-1,3 positions, producing free fatty acids and 2-monoacylglycerols. Palmitic acid in the form of monoacylglycerol is better absorbed than free palmitic acid, because the latter can bind calcium and magnesium ions, among others creating insoluble salts excreted in the faeces. From this point of view, the TAG structure of milk thistle oil is not suitable because palmitic acid is present mainly in the external positions of the TAG. However, this fatty acid does not occur in significant amounts in this oil [30,31].

Table 4. Distribution of the most important fatty acids in the external (*sn*-1,3s) and internal (*sn*-2) triacylglycerols (TAG) positions

Fatty acids	*sn*-2 [%]	*sn*-1,3 [%]
C16:0	1.16	12.12
C18:0	1.17	8.18
C18:1 n-9	28.12	26.98
C18:2 n-6	67.91	40.79
C20:0	0.64	5.32
C20:4 n-6	0.37	3.58

Source: own elaboration.

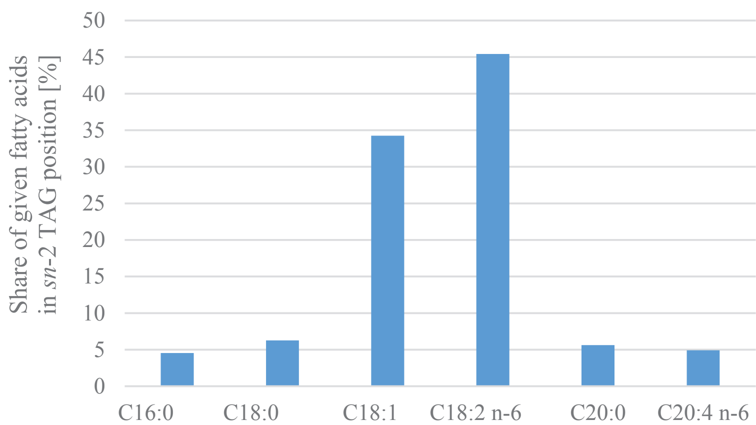

Figure 2. Percentage of a given fatty acid in sn-2 position of TAG of milk thistle seeds oil

Source: own elaboration.

Analysis the Total Phenolic Content (TPC)

The level of total phenolic content in the analysed commercial seed oils from *Silybum marianum* L. was on average 0.559 ±0.004 mg GAE/g. This value is lower than those reported by Parry et al. [32] and Dabbour et al. [33] for cold-pressed milk thistle seed oil (3.07 mg GAE/g and 1.16 mg GAE/g, respectively). The content of phenolic compounds in the tested oil was also significantly lower than oil pressed from several varieties of seeds grown in Tunisia, where the TPC ranged from 3.59 to 8.12 mg GAE/g [4]. According to Meddeb et al. [4], differences in TPC may be due to differences in the climate and plant growing conditions, such as soil, irrigation, and temperature. The content of phenolic compounds is correlated with the oxidative stability of the tested oil. A low TPC content (0.559 ±0.004 mg GAE/g) contributes to its low oxidative stability. This relationship is confirmed by research by Aparicio et al. [34], where the authors indicate a significant contribution of phenolic compounds (51%) to oxidative stability.

Antioxidant activity of milk thistle seed oil

The antioxidant activity of milk thistle seed oil was assessed using the DPPH method. Its value was approx. 19.13 ±1.60%. In general, the high antioxidant activity of vegetable oil could be attributed to its abundance of endogenous antioxidant ingredients such as tocopherol/tocotrienol, phytosterols, polyphenol, carotenoid, flavonoid, etc., which are able to scavenge free radicals and active oxygen. Zhang et al. [19] suggest that tocotrienol might play an important role in preventing milk thistle seed oil from oxidising.

CONCLUSIONS AND FUTURE PERSPECTIVES

Milk thistle is a plant with promising utility potential. Research on the physicochemical properties of milk thistle seed oil has shown that this oil is a valuable source of essential fatty acids, mainly linoleic and oleic. The ratio of unsaturated to saturated acids was approximately 4.31 ±0.22 and 3.95 ±0.02. The determined indicators of hypocholesterolemia/ hypercholesterolemia (h/H = 9.438) as well as atherogenicity (AI = 0.161) and thrombogenicity (TI = 0.899) showed that this oil is characterised by beneficial nutritional and health values and can be an attractive food product. The research carried out complements the existing knowledge of the physicochemical properties of this type of oil and can constitute a basis for further experiments using more advanced techniques.

Acknowledgements

Research equipment was purchased as part of the ‘Food and Nutrition Centre – modernisation of the WULS campus to create a Food and Nutrition Research and Development Centre (CŻiŻ)’ project co-financed by the European Union from the European Regional Development Fund under the Regional Operational Programme of the Mazowieckie Voivodeship for 2014–2020 (Project No. RPMA.01.01.00-14-8276/17).

REFERENCES

[1] **Sumara A., Stachniuk A., Montowska M., Kotecka-Majchrzak K., Grywalska E., Mitura P., Saftić Martinović L., Kraljević Pavelić S., Fornal E. 2023.** Comprehensive review of seven plant seed oils: chemical composition, nutritional properties, and biomedical functions. Food Reviews International 39(8): 5402–5422.

[2] **Yang R., Zhang L., Li P., Yu L., Mao J., Wang X., Zhang Q. 2018.** A review of chemical composition and nutritional properties of minor vegetable oils in China. Trends in Food Science & Technology 74: 26–32.

[3] **Fathi-Achachlouei B., Azadmard-Damirchi S. 2009.** Milk thistle seed oil constituents from different varieties grown in Iran. Journal of the American Oil Chemists’ Society 86: 643–649.

[4] **Meddeb W., Rezig L., Abderrabba M., Lizard G., Mejri M. 2017.** Tunisian milk thistle: An investigation of the chemical composition and the characterization of its cold-pressed seed oils. International Journal of Molecular Sciences 18(12), 2582: 1–13.

[5] **Tukan S.K., Takruri H.R., Al-Eisawi D.M. 1988.** The use of wild edible plants in the Jordanian diet. International Journal of Food Sciences and Nutrition 49: 1889–1895.

[6] **Seidler-Łożykowska K. 2010.** Hodowla i odmiany roślin zielarskich. Hodowla i Nasiennictwo 3: 16–20.

[7] **Hackett E.S., Twedt D.C., Gustafson D.L. 2013.** Milk thistle and its derivative compounds. A review of opportunities for treatment of liver disease. Journal of Veterinary Internal Medicine 27: 10–16.

[8] **Crocenzi F.A., Roma M.G. 2006.** Silymarin as a new hepatoprotective agent in experimental cholestasis: New possibilities for an ancient medication. Current Medicinal Chemistry 13: 1055–1074.

[9] **Shaker E., Mahmoud H., Mnaa S. 2010.** Silymarin, the antioxidant component and *Silybum marianum* extracts prevent liver damage. Food and Chemical Toxicology 48(3): 803–806.

[10] **Zheljazkov V., Zhalnov I., Nedkov N. 2006.** Herbicides for weed control in Blessed Thistle (*Silybum marianum*). Weed Technology 20:1030–1034.

[11] **Baranowska B., Kurzepa K., Marczak E., Szczucińska A., Lipkowski A. 2003.** Utylizacja odpadu nasion ostropestu plamistego II. Biologicznie czynne peptydy z odpadu nasion ostropestu plamistego. Rośliny Oleiste 24: 725–732.

[12] **Malekzadach M., Mirmazloum S., Mortazavi S., Panahi M., Angorani H. 2011.** Physicochemical properties and oil constituents of milk thistle (*Silybum marianum* Gaertn. Cv. Budakalaszi) under drought stress. Journal of Medicinal Plants Research 5(13): 2886–2889.

[13] **Qavami N., Naghdi Badi H., Labbafi M.R., Mehrafarin A.A. 2013.** Review on pharmacological, cultivation and biotechnology aspects of milk thistle (*Silybum Marianum* (L. Gaertn.). Journal of Medicinal Plants 12(47): 19–37.

[14] **Alemardan A., Karkanis A., Salehi R. 2013.** Breeding objectives and selection criteria for milk thistle (*Silybum marianum* L. Gaertn.) improvement. Not Bot Horti Agrobo 42(2): 340–347.

[15] **Aliyas I.M. 2017.** Wild milk thistle unique fatty plant. International Journal of Science and Research 6(1): 1227–1229.

[16] **Kazazis Ch.E., Evangelopoulos A.A., Kollas A., Vallianou N.G. 2014.** The therapeutic potential of milk thistle in diabetes. The Review of Diabetic Studies 11(2): 167–174.

[17] **Hadolin M., Skerget M., Knez Z., Bauman D. 2001.** High pressure extraction of vitamin E-rich oil from *Silybum marianum*. Food Chemistry 74(3): 355–364.

[18] **Marceddu R., Dinolfo L., Carrubba A., Sarno M., Di Miceli G. 2022.** Milk thistle (*Silybum marianum* L.) as a novel multipurpose crop for agriculture in marginal environments: A review. Agronomy 12, 729: 1–25.

[19] **Zhang Z.S., Wang S., Liu H., Li B.Z., Che L. 2020.** Constituents and thermal properties of milk thistle seed oils extracted with three methods. LWT – Food Science and Technology 126, 109282: 1–8.

[20] **PN EN ISO 5509:2001.** Animal and vegetable fats and oils – preparation of methyl esters of fatty acids. Polish Committee for Standardization, Warsaw, Poland.

[21] **Bryś J., Vaz I.F., Górska A., Wirkowska-Wojdyła M., Ostrowska-Ligęza E., Bryś A. 2017.** Use of GC and PDSC methods to characterize human milk fat substitutes obtained from lard and milk thistle oil mixtures. Journal of Thermal Analysis and Calorimetry 130(1): 319–327.

[22] **ISO 660:2009.** Animal and vegetable fats and oils – determination of acid value and acidity. International Organization for Standardization, Geneva, Switzerland.

[23] **ISO 3960:2007.** Animal and vegetable fats and oils – determination of peroxide value –iodometric (visual) endpoint determination. International Organization for Standardization, Geneva, Switzerland.

[24] **Hassanein M., Elshami S.M., Elmallah M.H. 2003.** Detailed studies on some lipids of *Silybum marianum* (L.) seed oil. Grasas y Aceites 54: 397–402.

[25] **Ayduğan A., Ok S., Yılmaz E. 2022.** Cold-pressed milk thistle seed oil: physico-chemical properties, composition and sensory analysis. Grasas Aceites 73(4): e481.

[26] **PN-EN ISO 3960:2017-03.** Vegetable and animal oils and fats. Determination of peroxide number (Reference Method). Polish Committee for Standardization, Warsaw, Poland.

[27] **Khan I., Khattak H.U., Ullah I., Bangash F.K. 2007.** Study of the physicochemical properties of Silybum marianum seed oil. Journal of The Chemical Society of Pakistan 29: 545–548.

[28] **Nyam K.L., Tan C.P., Lai O.M., Long K., Che Man Y.B. 2009.** Physicochemical properties and bioactive compounds of selected seed oils. Journal of Food Science and Technology 42: 1396–1403.

[29] **Siol M., Dudek A., Bryś J., Mańko-Jurkowska D., Gruczyńska-Sękowska E., Makouie S., Palani B.K., Obranović M., Koczoń P. 2024.** Chromatographic and thermal characteristics, and hydrolytic and oxidative stability of commercial pomegranate seed oil. Foods 13(9), 1370: 1–16.

[30] **Bryś J., Wirkowska M., Górska A., Ostrowska-Ligęza E., Bryś A., Koczoń P. 2013.** The use of DSC and FT-IR spectroscopy for evaluation of oxidative stability of interesterified fats. Journal of Thermal Analysis and Calorimetry 112: 481–487.

[31] **López-López A., Castellote-Bargalló A.I., Campoy-Folgoso C., Rivero-Urgel M., Tormo-Carnicé R., Infante-Pina D., López-Sabater M.C. 2001**. The influence of dietary palmitic acid triacylglyceride position on the fatty acid, calcium and magnesium contents of at term newborn faeces. Early Human Development 65: S83–S94.

[32] **Parry J., Hao Z., Luther M., Su L., Zhou K., Yu L.L. 2006.** Characterization of cold-pressed onion, parsley, cardamom, mullein, roasted pumpkin and milk thistle seed oils. Journal of the American Oil Chemists' Society 83: 847–854.

[33] **Dabbour I.R., Al-Ismail K.M., Takruri H.R., Azzeh F.S. 2014.** Chemical characteristics and antioxidant content properties of cold pressed seed oil of wild milk thistle plant grown in Jordan. Pakistan Journal of Nutrition 13: 67–78.

[34] **Aparicio R., Roda L., Albi M.A., Gutiérrez F. 1999.** Effect of various compounds on virgin olive oil stability measured by Rancimat. Journal of Agricultural and Food Chemistry 47: 4150–4155.

DOI: 10.22630/TPFP.2024.1.10139

ORIGINAL PAPER
Received: 01.12.2024
Accepted: 29.12.2024

INFLUENCE OF TEMPERATURE AND BREWING TIME ON THE TOTAL POLYPHENOL CONTENT IN TEA

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Abstract: Tea is a crucial source of antioxidant compounds – polyphenols – in a standard daily diet. On average, Poles drink 2–3 cups of tea daily. Its regular consumption may prevent cardiovascular and metabolic disorders. The objective of the study was to compare the total polyphenol content (TPC) in various types of tea infusions prepared at different temperatures (T = 70/100°C) and brewing times (t = 2/10 minutes). Organic and conventional black and green teas were tested. The TPC was measured using the Folin-Ciocalteu spectrophotometric method and expressed as gallic acid equivalents (mg GAE/g of the sample). Each tea extract was assayed three times independently. In the organic types of teas, the highest TPC was found in green tea (95.65 ±3.96 mg GAE/g) brewed at 100°C for 10 minutes, while the lowest in black tea (51.65 ±2.39 mg GAE/g) brewed at 70°C for 2 minutes. Among the conventional teas, the highest TPC was also noted in green tea (91.83 ±2.29 mg GAE/g) brewed at 100°C/10 minutes and the lowest in black tea (48.88 ±2.20 mg GAE/g) at 70°C/2 minutes. Longer brewing times increased TPC in organic teas (in both black and green) and in conventional black tea (p <0.05), whereas higher temperature increased TPC only in organic teas (black and green); (p <0.01).

Key words: antioxidant properties, black tea, brewing time, green tea, infusion, total polyphenol content

INTRODUCTION

Oxidation-reduction processes occur continuously within all living cells [1]. During some of these reactions, free radicals – reactive oxygen species – are formed. These can lead to the modification and damage of cell structures, including proteins, lipids and nucleic acids. Antioxidants are involved in reducing the production of excessive free radicals [2]. One group of compounds that possess antioxidant properties is polyphenols [3]. These natural substances, which are stored in various parts of plants, constitute an essential component of the standard daily diet [4]. The most important subgroups of polyphenols include phenolic acids and flavonoids. Among the phenolic acids, notable examples are caffeic acid, vanillic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, and gallic acid. On the other hand,

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flavonoids constitute a broad class of compounds, which can be further divided into subgroups such as flavonols (e.g., kaempferol, quercetin), flavones (e.g., apigenin), and flavanols, which include catechin and epicatechin [5].

Tea, as the most widely consumed beverage, is a significant source of polyphenols in the standard diet [6]. They demonstrate robust antioxidant properties that exert a protective effect against cardiovascular diseases and neurodegenerative disorders. They are also attributed to the ability to lower blood pressure, cholesterol and glucose levels in the blood. Regular polyphenol consumption may reduce the risk of cardiovascular disease due to their antioxidant effect on low-density lipoprotein (LDL). Additionally, some studies suggest that these compounds may protect the skin from the harmful effects of UV radiation [7, 8, 9].

Data indicate that the average Pole consumes approximately 2–3 cups of tea per day [10]. Tea consumed without the addition of sugar and milk does not provide calories and plays an important role in maintaining the body's hydration [11].

Leaves of the tea plant are characterised by their dark green colour and hard texture, while the flowers are soft and white. There are many varieties of tea. A typical example is the Chinese-origin *Camellia sinensis var. sinensis*, which demonstrates resistance to drought and cold. It is the most prevalent tea variety and the longest used in production [12, 13]. The raw materials to produce tea can be harvested up to several times a year, or even throughout the year in areas such as southern India, Ceylon and Indonesia. The traditional approach to harvesting involves laying the leaves on a surface such as a flat basket, bamboo mat or the floor. This is usually done under conditions of fresh air, sunlight (which provides additional stimulation and increases the number of catechins due to UV radiation) or in a ventilated room with a controlled temperature. The period of leaf wilting lasts between three and twelve hours, during which their moisture is reduced by 30–50%. Water loss facilitates leaf rolling [14]. At the time that the leaf is plucked from the tea branch, the oxidation in the leaf is already underway. It occurs gradually and without human intervention. The process is halted by the enzymes that initiate the oxidation, which are then deactivated [15]. The next point of production is rolling, during which the leaves are appropriately shaped and flattened. Most of these steps are now mechanised. However, high-quality tea is still twisted by hand. This stage influences the prolongation of the tea's shelf life. Next, the tea is sorted, classified, and packaged [16].

Worldwide, tea consumers prefer to drink black tea (76–78%), and next green tea (20–22%). Black tea undergoes the most extensive oxidation and fermentation processes compared to other types of tea. During the oxidation of black tea, catechins are transformed into theaflavins and thearubigins, which retain their antioxidant properties. These compounds, often referred to as tannins, contribute to the characteristic dark colour of black tea. Tannins may exhibit therapeutic effects on gastric and intestinal disorders. Green tea is particularly rich in catechins, which are a subclass of flavanol monomers, a type of flavonoids. The catechins in green tea comprise epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG). Apart from these antioxidant compounds, green tea also contains a small amount of vitamin C. The constituents of tea demonstrate antioxidant, antimutagenic, and anticarcinogenic properties. Regular consumption of green tea may prevent several types of cancer (lung, colon, oesophagus, oral cavity, stomach, small intestine, kidney, pancreas and mammary glands). Numerous epidemiological studies and clinical trials have indicated that green tea, and in some ways also black tea, may reduce the risk of various chronic diseases [17, 18, 19, 20].

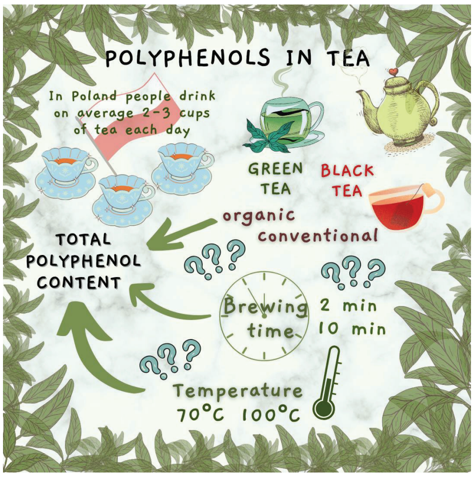

Figure 1. Do the type of tea, brewing time and temperatures influence the total polyphenol content in tea infusions?

Source: own elaboration.

It seems that the conditions under which the tea is brewed determine its antioxidant potential and the amount of antioxidative compounds in its infusion. The objective of the study was to compare the total polyphenol content (TPC) in organic and conventional types of tea infusions, prepared at different temperatures and brewing times (Fig. 1).

MATERIAL AND METHODS

This study included black and green tea in both options, organic and conventional. The organic tea, produced by *Dary Natury*, exhibited the requisite labelling indicative of organic certification. Conventional black tea (produced by *Oriental Delight*) and conventional green sencha (produced by *Sencha Classics*) were obtained from the same entity, *Basilur Tea*. The tea was provided in sachet form. The TPC of the tea was quantified according to the Folin-Ciocalteu (FC) spectrophotometric method [21] and expressed as mg of gallic acid equivalent per gram of the sample (mg GAE/g of the sample) using the equation obtained from the gallic acid calibration curve (R^2 >0.99); (Fig. 2). Determination of the TPC using this method is based on the reversible reaction of the reduction of molybdenum (VI) by polyphenols in an alkaline medium to molybdenum (V) contained in the FC reagent. The blue-coloured product formed shows an absorption maximum at λ = 745÷750 nm.

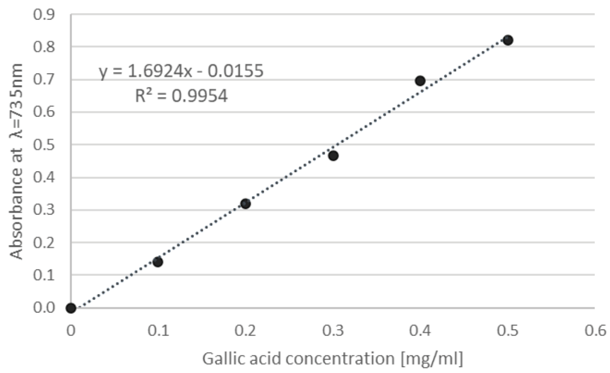

Figure 2. Calibration curve for a standard solution of gallic acid for determination of total polyphenol content

Source: own elaboration.

To prepare aqueous extracts, 1 g of tea was weighed into glass beakers (250 ml), which were then filled to a volume of 100 ml with demineralised water. The tea was infused with the water at temperatures (*T*) of 70°C and 100°C (immediately after boiling) for two different durations (*t*): 2 and 10 minutes. Each tea was brewed under four condition configurations:

- 70°C for 2 minutes,
- 70°C for 10 minutes,
- boiling water for 2 minutes,
- boiling water for 10 minutes.

After the designated brewing time, the infusions were decanted. The resulting extracts were then transferred to new beakers, taking 5 ml of the sample from each infusion.

In brief, 5 ml of the sample with 2 ml of distilled water was mixed with 50 µl of the tea extract and 250 µl of Folin-Ciocalteu solution. After 3 minutes, 750 µl of Na_2CO_3 solution was added and vortexed. The blank was prepared using the same process but with 50 µl of distilled water instead of the sample. The tubes were covered with aluminium foil and placed in a water bath at 40°C for 30 minutes. After incubation, the absorbance was measured at a wavelength equal to 735 nm using a UV-Vis spectrophotometer (Pharmacia LKB Ultrospec III). Each measurement was repeated three times independently [22].

Statistical analysis was performed using the Statistica 13.3 software. Analysis of variance (ANOVA) was conducted to evaluate the influence of the different temperatures and tea brewing times on the TPC. To assess the significance of differences between the individual samples of tea, post-hoc tests were used. The significance level was set at p <0.05.

RESULTS AND DISCUSSION

The results of the TPC determinations are presented in Figures 3 and 4.

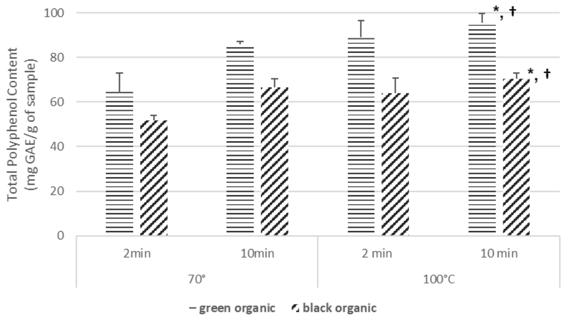

Figure 3. Comparison of total polyphenol content in organic tea brewed for 2 and 10 minutes at temperatures of 70°C and 100°C. Results are presented as mean ± standard deviation, * – *p* <0.05, † – *p* <0.01

Source: own elaboration.

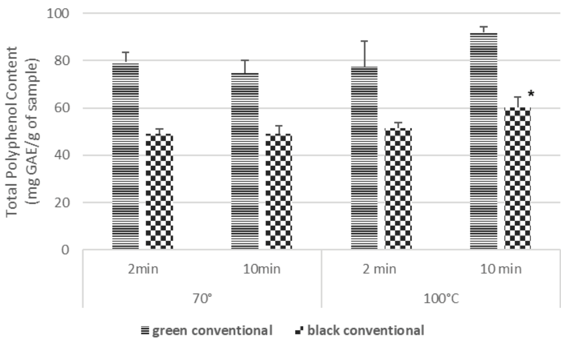

Figure 4. Comparison of total polyphenol content in conventional tea brewed for 2 and 10 minutes at temperatures of 70°C and 100°C. Results are presented as mean ± standard deviation, * – *p* <0.05

Source: own elaboration.

Of the organic tea varieties, the highest TPC was found in the green tea, amounting to 95.65 ±3.96 mg GAE/g of the sample, when brewed at 100°C for 10 minutes. In contrast, the lowest TPC was recorded in the black tea, with a value of 51.65 ±2.39 mg GAE/g of the sample, under brewing conditions of 70°C for 2 minutes (Fig. 3). Tea brewed with boiling water (for both times: 2 and 10 min) showed a higher TPC than those brewed at $T = 70$°C. Tea steeped for 10 minutes (in both T: 70°C and boiling water) had a higher TPC compared to those brewed for a shorter time across all organic tea types: black and green.

In the conventional types of tea, the highest TPC was also identified in green tea, reaching 91.83 ±2.29 mg GAE/g of the sample, when brewed at 100°C for 10 minutes. Conversely, the lowest TPC was found in black tea, with a value of 48.88 ±2.20 mg GAE/g of the sample, brewed at 70°C for 2 minutes (Fig. 4).

When analysing the differences in polyphenol content in conventional tea (depending on brewing temperature and time), it was found that conventional green tea had the highest polyphenol content (under conditions of $T = 100$°C, $t = 10$ minutes), while conventional black tea had the lowest (at $T = 70$°C, $t = 2$ minutes). Both conventional black and green tea brewed with boiling water had higher polyphenol contents compared to those brewed at 70°C.

Green tea (both organic and conventional) exhibited a higher TPC compared to black tea. The longer brewing time ($t = 10$ minutes) increased the TPC in organic tea (in both black and green) and in conventional black tea ($p < 0.05$), whereas the higher temperature increased the TPC only in the organic teas (black and green) ($p < 0.01$).

As stated above, according to the statistics on tea consumption among people in Poland, on average, 2–3 cups of tea are consumed daily. Based on our results, we can conclude that drinking three cups (we assumed that 1 cup is defined as 1.5 g of tea brewed with about 150 ml of water) of organic green tea daily (brewed for 2 minutes at 70°C) will provide the organism with 290.7 mg of polyphenols daily. If the same tea is brewed with water at 100°C for 10 minutes, the supplied amount of polyphenols increases to 430.4 mg. If the individual usually drinks organic black tea (brewed for 10 minutes at 100°C), they may consume 316.4 mg of polyphenols.

The polyphenol contents of black and green teas reported in the literature are within very wide limits. Comparison of the results obtained is hampered by the use of different solvents (water, methanol), the use of various methods for measuring polyphenols, but also various methods for presenting the results in terms of gallic acid or catechin equivalents per 1 g of tea dry weight or volume of 100 or 200 cm^3. It is also necessary to take into account the variability resulting from the specific characteristics of different tea cultivars, production, and storage conditions.

The Folin-Ciocalteu method used in this study is the most widely applied and convenient international standard method (ISO 14502-1:2005) to determine the total phenolic content in foods, herbs, and other plant extracts [23]. The disadvantage of this method lies in the fact that it is based on the oxidation of -OH groups in tea polyphenols, which can easily oxidise some non-tea polyphenol compounds, making the theoretical result higher than the actual value. More accurate quantitative analysis of functional constituents in tea is possible with near-infrared (NIR) spectroscopy with non-destructive detection and electrochemical sensor technology capable of rapid on-site detection. Moreover, enhanced liquid chromatography (LC) and high-resolution mass spectrometry (HRMS) are used for the simultaneous determination of multiple polyphenols and the identification of new polyphenols [24]. Although the latter methods are characterised by high sensitivity, precision and resolution, they are more costly and require specialised, expensive equipment.

In the study conducted by Cody [24], the polyphenol content of ten different tea infusions was determined using Matrix-Assisted Ionisation in Vacuum (MAIV) mass spectrometry (MS). The analysis encompassed nine infusions derived from *Camellia sinensis*, including three green teas, two black teas, two oolong teas, jasmine tea, and white tea, as well as one rooibos infusion. The polyphenol content in tea infusions ranged from 19.2 to 108.6 mg/100 mL across the different tea types, with green teas generally exhibiting higher polyphenol concentrations compared to black teas. These findings align with our results, where both the organic and conventional green teas showed the highest TPC.

In the study by McAlpine et al., eight types of tea were characterised in terms of TPC and antioxidant capacity with respect to brewing time [25]. As in the present study, the TPC in the tea infusions was determined using the F-C method. Tea samples were brewed for durations ranging from 1 to 10 minutes, in 1-minute intervals and the TPC was subsequently measured, which increased with longer brewing times; however, most of the polyphenols observed after 10 minutes were already extracted within the first 5 minutes, regardless of the type of tea. Green teas demonstrated higher TPC values than black ones, similar to the findings of the current study. For instance, in the referenced study, the TPC for sencha (a type of green tea) brewed for 2 minutes was 52.42 ±6.90 mg GAE/g of tea, while for 10 minutes it was 112.37 ±3.03 mg GAE/g. Similarly, in the current study, the TPC for organic green tea increased with the longer brewing time, reaching, for example, 64.59±8.49 mg GAE/g of the sample at T= 70°C for t = 2 minutes, and 85.79 ±1.44 mg GAE/g after 10 minutes of brewing. A comparable trend was observed for black teas.

The study by Vinci et al. investigated the influence of various brewing parameters (type of water, brewing time, temperature, and pH) on the TPC and antioxidant activity of green and black tea infusions [26]. An increase in the TPC was observed with longer brewing times for each type of water. As in the present study, higher TPC values were consistently recorded for green tea. Black tea demonstrated a comparable trend to green tea, with a higher TPC observed in infusions brewed at T = 100°C compared to T = 80°C (in our study, T = 70°C). The parameters that had the most significant impact on the TPC were the brewing time and the type of water used for infusion preparation. Similarly to our findings, the brewing time had a significant effect on the TPC levels in the tea samples.

In the study by Mehrabi et al., the impact of various tea preparation techniques on the content of bioactive compounds, antioxidant capacity, antibacterial properties, and polyphenol bioavailability in green, black, and oolong tea infusions was compared [27]. All tea types demonstrated an increase of TPC over longer brewing times. Consistent with our findings in this study, higher TPC values were reported for green tea, regardless of the preparation method, compared to black tea. The study highlighted the significant influence of the oxidation process on the tea's properties. Microwave-assisted brewing was identified as the most effective technique for maximising the bioactive compound content and bioavailability, which was attributed to rapid and uniform heating, which facilitates efficient extraction while minimising degradation. While all preparation methods showed a positive correlation between the TPC and antioxidant and antibacterial activities, the traditional steeping method resulted in the highest release of phenolic and flavonoid compounds after 10 minutes of brewing. This suggests that the brewing parameters, such as time and temperature, significantly influence the extraction efficiency of phenolic compounds, emphasising the importance of optimising these factors for maximising health-related benefits from tea infusions.

A Polish study by Dmowski et al., also using the Folin-Ciocalteu method, assessed the impact of the steeping time and the degree of fragmentation of black tea on its antioxidant properties [22]. The tea was brewed at approximately 90°C. The results presented in the study indicated that steeping time affected the level of polyphenolic compounds. Infusions brewed for 15 minutes exhibited higher values (average content in the analysed samples was 239.57 mg GAE/100 ml) compared to those brewed for 3 minutes (67.70 mg GAE/100 ml). Furthermore, it was noted that the degree of fragmentation and the brand of tea were not statistically significant factors. In the present study, the steeping time also influenced the polyphenol content. In this study, the teas steeped for 10 minutes contained more polyphenols than those steeped for 2 minutes (for instance, in organic black tea, the polyphenol content after 10 minutes of brewing was higher at 70.30 ±2.74 mg GAE/g of the sample, compared to 63.92 ±6.72 mg GAE/g of the sample for the 2-minute infusion, both at a constant brewing temperature of T = 100°C).

The antioxidant properties of four green teas, depending on the extraction temperature, were compared in the study by Ramirez-Aristizabal et al. [28]. Various methods were employed for the measurements, including the Folin-Ciocalteu method. The brewing time for the tea was set at 5 minutes. Higher temperatures (80°C) for tea brewing, compared to lower (in this case, it was at room temperature, 25°C), resulted in a greater polyphenol content (for example, in one of the green teas, the TPC at T = 80°C was 55.06 ±1.03 mg GAE/g of sample, whereas at T = 25°C it was 14.63 ±0.53 mg GAE/g of the sample). In our study, higher brewing temperatures also resulted in an increase in the TPC. The TPC of organic green tea brewed for 2 minutes at 70°C was 64.59 mg GAE/g of the sample, whereas, at 100°C, the TPC increased to 88.94 mg GAE/g of the sample.

In contrast, a study by Pintać et al. compared the phenolic profiles and antioxidant activities expressed as real serving concentrations of the most popular plant-based drinks: coffee, tea, and wine [29]. Similar to our study, the TPC of green tea reached higher values than black tea. For example, looking at teas from the same manufacturer: green tea contained 340 ±26.2 mg GAE/serving, while black tea contained 164 ±0.42 mg GAE/serving. All three categories of beverages demonstrated a substantial concentration of phenolic compounds, exhibiting significant antioxidant potential. Nevertheless, the green tea infusions displayed markedly superior antioxidant activity. Based on the findings, moderate consumption of coffee and red wine may also be recommended as a valuable source of polyphenols. In our study, conventional green tea brewed for 2 minutes at 70°C achieved a TPC of 79.56 mg GAE/g of the sample, while conventional black tea, under the same brewing conditions, exhibited a TPC of 48.88 mg GAE/g of the sample.

Our study provides interesting information regarding the amount of polyphenol in tea and influencing factors, but it is not free from some limitations. Further studies should involve a larger number of samples in order to confirm the present findings. Other, more accurate, modern methods could be used to carefully assess the contributions of individual polyphenols to the TPC. Chromatographic techniques, particularly High-Performance Liquid Chromatography (HPLC), remain the standard for the qualitative and quantitative analysis of individual polyphenolic compounds in tea, while spectrophotometric techniques continue to be widely employed. These methods, when used in combination, would provide a more comprehensive evaluation of the polyphenolic profile in tea, supporting researchers in understanding the relationship between tea's composition and its potential health benefits.

Despite the increasing adoption of advanced methods, such as near-infrared spectroscopy and electrochemical sensors, the Folin-Ciocalteu method remains the most commonly used approach for determining TPC. As noted by Sun et al. [24], this method is favoured for its stability, reliability, and simplicity. Other comparative studies between various organic and conventional teas from different producers would also be desired.

CONCLUSIONS AND FUTURE PERSPECTIVES

A higher brewing temperature resulted in an increased polyphenol content in infusions from both organic (green and black) teas, as well as from conventional teas. However, a longer tea brewing time led to an increase in polyphenol levels, but only in the case of organic tea (both black and green). It is worth paying attention not only to the brand of tea but also to its brewing parameters, which certainly translates into the amount of polyphenols in the daily diet.

REFERENCES

[1] **Sies H. 2015.** Oxidative stress: a concept in redox biology and medicine. Redox Biology 4: 180–183.

[2] **Jopkiewicz S. 2018.** Stres oksydacyjny cz. II. Profilaktyka powstania uszkodzeń wolno rodnikowych. Medycyna Środowiskowa 21(2): 53–59.

[3] **Puzanowska-Tarasiewicz H., Kuzmicka L., Terasiewicz M. 2010.** Antyoksydanty a reaktywne formy tlenu. Bromatologia i chemia toksykologiczna 63(1): 9–14.
[4] **Gryszczyńska B., Iskra M. 2008.** Współdziałanie antyoksydantów egzogennych i endogennych w organizmie człowieka. Nowiny Lekarskie 77(1): 50–55.
[5] **Szwaczka P., Grembecka M., Brzezicha-Cirocka J., Szefer P. 2017.** Substancje o charakterze antyoksydacyjnym w suplementach diety na bazie herbaty. Bromatologia i Chemia Toksykologiczna 50(4): 311–317.
[6] **Yan Z., Zhong Y., Duan Y., Chen Q., Li F. 2020.** Antioxidant mechanism of tea polyphenols and its impact on health benefits. Animal Nutrition 6(2): 115–123.
[7] **Ilow R., Regulska-Ilow B., Rózańska D., Misiewicz D., Grajeta H., Kowalisko A., Biernat J. 2012.** Assessment of dietary flavonoid intake among 50-year-old inhabitants of Wroclaw in 2008. Advances in Clinical and Experimental Medicine 21(3): 353–362.
[8] **Koch W. 2016.** Napary z czarnej herbaty jako główne źródło związków polifenolowych w racjach pokarmowych studentów. Bromatologia i Chemia Toksykologiczna 49(3): 308–312.
[9] **Hu S., Zhang X., Chen F., Wang M. 2017.** Dietary polyphenols as photoprotective agents against UV radiation. Journal of Functional Foods 30: 108–118.
[10] **Piela A. 2015.** Zwyczaj picia kawy i herbaty odzwierciedlony w polskim słownictwie i frazeologii. LingVaria (1): 141–152.
[11] **Bienia B., Uram-Dudek A., Dykiel M., Krochmal-Marczak B., Sawicka B. 2022.** Właściwości przeciwutleniające wybranych herbat zielonych. HERBALISM 5(1): 32–40.
[12] **Miazga-Sławińska M., Grzegorczyk A. 2014.** Herbaty – rodzaje, właściwości, jakość i zafałszowania. Kosmos. Problemy Nauk Biologicznych 63(3): 473–379.
[13] **Cichoń Z., Miśniakiewicz M. 2005.** Analiza jakości czarnych herbat liściastych. Zeszyty Naukowe (678): 103–127.
[14] **Aaqil M., Peng C., Kamal A., Nawaz T., Zhang F., Gong J. 2023**. Tea Harvesting and Processing Techniques and Its Effect on Phytochemical Profile and Final Quality of Black Tea. A Review. Foods 12(24): 4467.
[15] **Zhou J., Gao S., Du Z., Xu T., Zheng C., Liu Y. 2024.** The Impact of Harvesting Mechanization on Oolong Tea Quality. Plants (Basel) 13(4): 552.
[16] **Plust D., Czerniejewska-Surma B., Domiszewski Z., Bienkiewicz G. 2011.** Zawartość polifenoli, właściwości przeciwutleniające oraz zdolności redukujące naparów herbat białych liściastych. Folia Pomeranae Universitatis Technologiae Stetinensis seria Agricultura, Alimentaria, Piscaria et Zootechnica 286(18): 47–52.
[17] **Brimson J.M., Prasanth M.I., Kumaree K.K., Thitilertdecha P., Malar D.S., Tencomnao T., Prasansuklab A. 2022.** Tea Plant (*Camellia sinensis*): A Current Update on Use in Diabetes, Obesity, and Cardiovascular Disease. Nutrients 15(1): 37.
[18] **Sharma A., Dutta P.P. 2018.** Scientific and Technological Aspects of Tea Drying and Withering: A Review. Agricultural Engineering International: CIGR Journal 20(4): 210–220.
[19] **Kaur A. 2015.** Estimation and comparison of total phenolic and total antioxidants in green tea and black tea. Global Journal of Biochemistry and Biotechnology 4(1): 116–120.
[20] **Skotnicka M., Chorostowska-Wynimko J., Jankun J., Skrzypczak-Jankun E. 2011.** The black tea bioactivity: an overview. Central European Journal of Immunology 36(4): 284–292.
[21] **Saidan A., Azakir B., Neugart S., Kattour N., Sokhn E.S., Osaili T.M., Darra N.E. 2024.** Evaluation of the Phenolic Composition and Biological Activities of Six Aqueous Date (*Phoenix dactylifera* L.) Seed Extracts Originating from Different Countries: A Comparative Analysis. Foods 13(1): 126.
[22] **Dmowski P., Śmiechowska M., Sagan E. 2014.** Wpływ czasu parzenia i stopnia rozdrobnienia herbaty czarnej na barwę naparu i jego właściwości przeciwutleniające. Żywność. Nauka. Technologia. Jakość 5(96): 206–216.
[23] **ISO 14502-1:2005.** Determination of substances characteristic of green and black tea. Part 1: Content of total polyphenols in tea – Colorimetric method using Folin-Ciocalteu reagent. (https://www.iso.org/standard/31356.html, access: 19.12.2024).
[24] **Sun M.F., Jiang C.L., Kong Y.S., Luo J.L., Yin P., Guo G.Y. 2022.** Recent advances in analytical methods for determination of polyphenols in tea: a comprehensive review. Foods 11(10): 1425.
[25] **McAlpine M.D., Ward W.E. 2016.** Influence of Steep Time on Polyphenol Content and Antioxidant Capacity of Black, Green, Rooibos, and Herbal Teas. Beverages 2(3): 17.
[26] **Vinci G., D'Ascenzo F., Maddaloni L., Prencipe S.A., Tiradritti M. 2022.** The Influence of Green and Black Tea Infusion Parameters on Total Polyphenol Content and Antioxidant Activity by ABTS and DPPH Assays. Beverages 8(2): 18.
[27] **Mehrabi M., Amiri M., Razavi R., Najafi A., Hajian-Tilaki A. 2025.** Influence of varied processing methods on the antioxidant capacity, antibacterial activity, and bioavailability of Iranian black, oolong, and green leafy teas. Food Chemistry 464(2): 141793.
[28] **Ramirez-Aristizabal L.S., Ortiz A., Restrepo-Aristizabal M.F., Salinas-Villada J.F. 2017.** Comparative study of the antioxidant capacity in green tea by extraction at different temperatures of four brands sold in Colombia. Vitae 24(2): 132–145.
[29] **Pintać D., Bekvalac K., Mimica-Dukić N., Raseta M., Andelić N., Lesjak M., Orcić D. 2022.** Comparison study between popular brands of coffee, tea and red wine regarding polyphenols content and antioxidant activity. Food Chemistry Advances 1: 100030.

DOI: 10.22630/TPFP.2024.1.10031

ORIGINAL PAPER
Received: 15.10.2024
Accepted: 31.01.2025

SCIENCE AND TECHNOLOGY PARTNERSHIP: APPLICATION OF COMPUTATIONAL FLUID DYNAMICS TO PREDICT THE TURKEY MEAT PROTEIN DENATURATION PROCESS IN THE CONTEXT OF A HEALTHY DIET FOR CHILDREN AND ADOLESCENTS

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Abstract: In the current era marked by increased consumer awareness and advancements in food technology, the quality of turkey meat has emerged as a focal point, particularly in promoting healthy diets for children and adolescents. As parents seek nutritious and appealing food options for younger consumers, understanding protein denaturation becomes critical for enhancing meat texture, juiciness, and overall sensory experience. This study explores the application of Computational Fluid Dynamics to predict and optimise the denaturation of turkey meat proteins during thermal processing. By utilising CFD, this research models the heat and mass transfer dynamics involved in cooking turkey meat, providing insights that can optimise the cooking conditions to preserve nutritional value while improving the sensory qualities. The results indicated optimal thermal treatment conditions – 161.28°C, 61.31% humidity, and 17.58 rpm fan speed. Laboratory validations confirmed that the predicted denaturation of myosin and actin aligned closely with experimental results, underscoring the efficacy of CFD as a predictive tool. Moreover, no statistically significant discrepancies were observed in collagen denaturation between the predicted and experimental results (P >0.05), further demonstrating the accuracy of the model. Overall, this work illustrates the potential of CFD in food science, enabling the development of high-quality, safe, and sustainable turkey meat products that fulfil the nutritional needs of children and adolescents.

Key words: Computational Fluid Dynamics (CFD), Protein Denaturation, Turkey Meat Processing, Healthy Diet for Children, Thermal Process Optimisation, Nutritional Quality in Meat

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INTRODUCTION

Understanding the quality of turkey meat is crucial for promoting healthy diets, particularly for children and adolescents. Turkey meat is valued for its lean protein content and health benefits, making it a recommended component of balanced diets for younger consumers. However, achieving high standards of nutritional quality, sensory appeal, and safety in turkey meat requires an in-depth understanding of the thermal processes that influence protein denaturation during cooking. This phenomenon significantly impacts the meat texture, juiciness, and overall appeal, which are critical factors in encouraging healthier eating habits [1–3].

Computational Fluid Dynamics (CFD) offers an innovative approach to modelling and optimising the thermal processing of turkey meat. By simulating heat and mass transfer, CFD provides insights into how temperature and cooking time affect protein structure and quality. This technology enables precise control over heat distribution and minimising protein degradation while preserving nutritional value and sensory qualities [4, 5]. Optimising these processes not only ensures high-quality meat products but also aligns with dietary guidelines for children and adolescents by maintaining digestibility and reducing the content of undesirable compounds [6].

CFD also supports sustainability and food safety initiatives. By optimising the cooking conditions, it reduces energy consumption, minimises waste, and ensures uniform cooking, which helps eliminate the risks associated with undercooked meat while maintaining its sensory attributes [7, 8]. These advancements demonstrate the potential of CFD to enhance the quality and healthfulness of turkey meat, contributing to broader goals of promoting sustainable and nutritious food options for younger generations [9, 10].

This study aims to explore the application of CFD in predicting and optimising the denaturation process of turkey meat proteins during thermal processing. It also examines potential benefits of this technology in enhancing food safety, sustainability, and energy efficiency, supporting dietary and environmental objectives.

MATERIAL AND METHODS

The experiment was carried out in several phases:

- in the first step, an experimental model was developed using the Design-Expert program,
- the second phase was a simulation of the heat treatment process using CFD,
- the third stage was roasting optimisation using RSM (response surface methodology),
- the final stage was verification of the predicted results using laboratory tests.

Experiment design

Three design variables – temperature, humidity, and fan rotation speed – were chosen as quantitative independent factors to evaluate their effects on the denaturation levels of myosin, collagen, actin, and cooking loss. These variables were tested in the following ranges: temperature from 120°C to 200°C, humidity from 0% to 75%, and fan rotation speed from 0 to 1,400 rpm. The parameter ranges were established through a combination of literature review and preliminary experiments [4]. To assess the outcomes, the Design-Expert version 11 software (Stat-Ease, Inc., USA) was employed, generating 20 experimental runs (as shown in Table 1). A quadratic equation was used to model the interactions between variables and responses, with the model's central point repeated six times for validation.

Table 1. Experimental design used for CFD simulations

Run	Heat treatment parameters		
	Temperature [°C]	Humidity [%]	Fan rotation [rpm]
1	120	0	0
2	200	0	0
3	120	75	0
4	200	75	0
5	120	0	1,400
6	200	0	1,400
7	120	75	1,400
8	200	75	1,400
9	120	37.5	700
10	200	37.5	700
11	160	0	700
12	160	75	700
13	160	37.5	0
14	160	37.5	1,400
15 C	160	37.5	700
16 C	160	37.5	700
17 C	160	37.5	700
18 C	160	37.5	700
19 C	160	37.5	700
20 C	160	37.5	700

C – central points

Source: own elaboration

Heat treatment process using CDF simulation. CFD Description and Implementation

The Ansys v.19.0 software was utilised to develop a three-dimensional model of the testing apparatus and to simulate the thermal treatment process. This simulation followed the methodology described by Szpicer et al. (2022), with some modifications made for the study [11]. The scheme of the studied system is shown in Figure 1. Key factors affecting the thermal properties of food products include their fundamental composition, which consists of the amounts of protein, fat, carbohydrates, and ash. The conduction coefficient (λ) and specific heat (Cp) of the raw material are critical in determining these properties. The basic composition of turkey breast meat can vary due to factors such as age, developmental stage, and activity levels [12–15]. The thermophysical properties of turkey meat, such as the thermal conductivity (λ), specific heat (Cp), and density, were treated as temperature-dependent variables. These properties were calculated using the equations proposed by Choi and Okos (1986), with adjustments based on the meat's composition. Furthermore, water evaporation during cooking was modelled using Fick's law of diffusion, and its impact on the local thermal conductivity and specific heat was incorporated into the simulation [16].

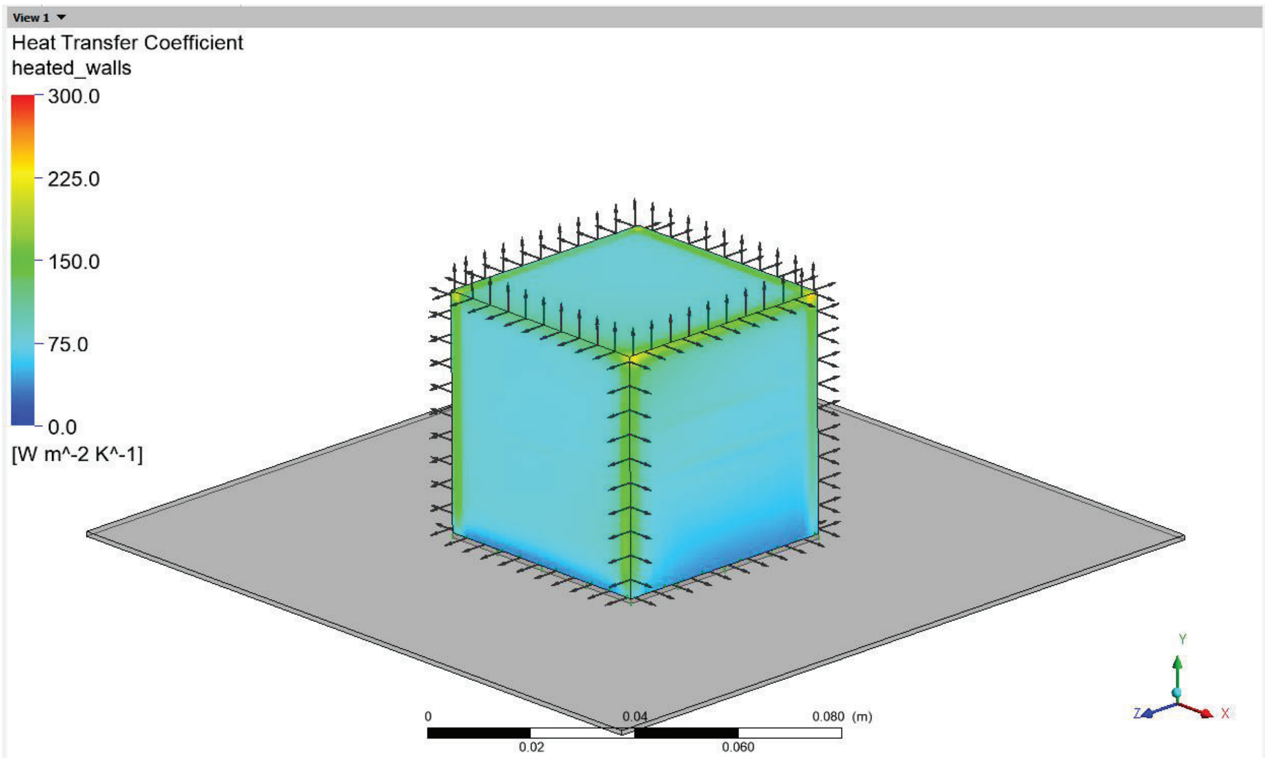

Figure 1. Scheme of the studied system

Source: own elaboration.

For the simulation, input data were sourced from the composition analysis conducted by Gantner et al. (2017), which reported the following values: 77.03 ±0.21% water, 1.98 ±0.49% fat, 19.03 ±0.23% protein, 1.46 ±0.45% connective tissue, and 1.61 ±0.03% ash [17]. The objective of this research was to determine the thermal penetration coefficient to calculate the denaturation extent of individual proteins and the resulting cooking loss. Temperature and enthalpy data for each protein type were gathered using differential scanning calorimetry (DSC): myosin at 61.5°C, collagen at 67°C, and actin at 80.5°C, with corresponding enthalpies of 3.85 J/gK, 3.84 J/gK, and 3.93 J/gK, respectively [15]. For the simulation, a 3D model of a combi steamer (CPE 110, Convect-Air Professional, Küppersbusch, Germany) was created following the methodology specified by Szpicer et al. (2024) [18]. The model incorporated a heat source (heater), fan, steam generator, and GN container rack. A cube of turkey breast meat (50 × 50 × 50 mm) was placed on a GN ½ tray (530 × 325 × 20 mm) inside the oven. The simulated roasting duration was established at 2,000 seconds, assuming the turkey breast meat had a homogeneous microstructure and isotropic properties regarding mass and heat, without accounting for transport and fat loss. The forced convection was characterised by the global heat transfer coefficient, and water diffusion was modelled using Fick's law of diffusion with a global diffusion coefficient. Cooking loss from water squeezing was considered, while thermal contraction and shape changes were neglected, keeping the sample volume constant throughout the roasting process [10, 11 ,19].

NUMERICAL MODEL

Analysed space

The model comprised three distinct domains. The initial domain encompassed the fluid surrounding the sleeve-shaped rotor, which operated at a designated rotational speed. This configuration facilitated the simulation of the rotor's rotation within the chamber, with the blades represented as surfaces. The second domain represented the chamber area where air circulation was induced by the rotating rotor. Lastly, the third domain included two solid components: the sample insert and the support plate.

Mesh

A hexa structural mesh was applied to the rotor space, incorporating surface compaction for the rotor blades to enhance the representation of the flow. For the chamber, a tetra/prism mesh was utilised, with prismatic elements strategically placed, such as along the meat and plate walls, for heat transfer analysis. A hexa mesh was chosen for the turkey breast meat and plate. The model specifications were as follows: rotor space – 152,880 hexes and 177,702 nodes; chamber – 339,867 tetras, 100,385 prisms and 120,714 nodes; meat and plate – 64,372 hexes and 73,325 nodes.

Materials

In the computations, the materials used included a blend of air and water vapour, turkey meat as the used material, and stainless steel (AISI 304). The initial temperature of the solids was set at 23°C.

MATERIAL DATA

Turkey breast meat

The determination of the conduction coefficient (λ) relied on Eq. (1) following the description by Choi and Okos, considering the essential composition of the sample [16].

$$\lambda = 0.205x_c + 0.20x_p + 0.175x_f + 0.135x_a + 0.61x_w \quad (1)$$

where:
x – mass fraction of the food ingredient,
indexes:
c – carbohydrates,
p – proteins,
f – fat,
a – ash,
w – water.

The computation of specific heat (C_p) was derived from Eq. (2) following the explanation provided by Singh and Heldman, considering the fundamental composition of the samples [20].

$$C_p = 1.424x_c + 1.549x_p + 1.675x_f + 0.837x_a + 4.187x_w \quad (2)$$

where:
x – mass fraction of the food ingredient,
indexes:
c – carbohydrates,
p – proteins,
f – fat,
a – ash,
w – water.

Stainless steel (AISI 304)
Conduction coefficient (λ) – 60.5 W/mK
Specific heat (C_p) – 434 J/kgK.
Air – Air Ideal Gas:

The ideal gas equation of state can be applied for the computation of different gas properties according to Eq. (3).

$$pV = nRT \tag{3}$$

where:
p – absolute pressure of the gas,
V – volume of the gas,
n – amount of substance of gas (also known as number of moles),
R – ideal, or universal, gas constant, equal to the product of the Boltzmann constant and the Avogadro constant,
T – absolute temperature of the gas.

Water vapour – Redlich-Kwong Dry Steam:

Pressure and molar volume of dry steam were calculated using the Redlich–Kwong equation (Eq. 4) to accurately model heat and mass transfer conditions in the CFD simulations [21]:

$$p = \frac{RT}{V_m - b} - \frac{a}{\sqrt{T} V_m \left(V_m + b\right)} \tag{4}$$

where:
p – gas pressure,
R – gas constant,
T – temperature,
V_m – molar volume (V/n),
a – constant that corrects for the attractive potential of molecules,
b – constant that corrects for the volume.

The gas used in the experiment was a blend of steam and air, with the mixture's composition determined by the mass fractions of its individual components.

Humidity

$$\log e^* = -7.90298\left(\frac{T_{st}}{T} - 1\right) + 5.02808\log\left(\frac{T_{st}}{T}\right) - 1.3816\times10^{-7}\left(10^{11.344\left(1-\frac{T}{T_{st}}\right)} - 1\right) + 8.1328\times10^{-3}\left(10^{-3.49149\left(\frac{T_{st}}{T}\right)} - 1\right) + \log e^*_{st} \tag{5}$$

where:
log refers to the logarithm in base 10,
e* – saturation water vapour pressure (hPa),
T – absolute air temperature in Kelvins,
T_{st} – steam-point (i.e. boiling point at 1 atm.) temperature (373.15 K),
e^*_{st} – e* at the steam-point pressure (1 atm = 1,013.25 hPa).

Using Eq. (6), the mass fraction of water vapour in the mixture was established by considering the saturation pressure at the provided temperature and the designated humidity.

$$X = 6.222\frac{\phi Ps}{1013.25 + \phi Ps} \tag{6}$$

$$gH_2O = \frac{X}{X+1}$$

Definition of the solver

The SST turbulence model solver parameters were configured with a convergence criterion of 10e-4 and 600 iterations. Before finalising the mesh size and solver parameters, a mesh independence study was conducted to ensure the robustness of the model. Several mesh configurations were tested, gradually increasing the number of elements, while observing the impact on the key output parameters, such as the temperature distribution, heat transfer coefficients, and protein denaturation levels. The selected mesh (152,880 hex elements for the rotor space, 339,867 tetrahedral elements for the chamber, and 64,372 hex elements for the meat and plate) provided a balance between computational cost and accuracy. Further refinement resulted in negligible changes to the output parameters (<1%). In addition, an adaptive time-stepping approach was configured, with a range of 0.1 to 10 seconds and 2–5 iterations per step. This setup was chosen to address the transient nature of the heat treatment process. Smaller time steps were applied during periods of rapid temperature changes, such as the initial heating phase, while larger steps were used when temperature gradients had stabilised. This approach ensured numerical stability, accurate results, and optimised computational efficiency.

The solver work type was designated as Double Precision. The choice of the Shear Stress Transport (SST) turbulence model was based on its flexibility, effective handling of boundary layers, relatively low computational complexity, and widespread use in the field of CFD. For the transient state solver definition, the adaptive time step was established with 2–5 iterations per step, a time step range of 0.1–10 seconds, and a process duration of 2,000 seconds.

Validation of the Computational Model

In this study, a detailed validation of the Computational Fluid Dynamics (CFD) model was conducted to confirm its reliability in predicting temperature distributions within turkey breast samples during thermal processing. This step was crucial for ensuring the model's ability to accurately simulate protein denaturation, which is driven by temperature changes.

Experimental Setup

To validate the model, turkey breast samples (50 × 50 × 50 mm) were thermally treated in a combi-steam oven (Küppersbusch CPE-110, Germany) at: 160°C, 37.5% humidity, and 1,400 rpm fan speed. Temperature measurements were taken using Type-K thermocouples (Ellab TrackSense Pro, Denmark) placed at three key locations within the sample:

- Core: Geometric centre of the meat cube.
- Midpoint: Halfway between the core and the surface.
- Surface: Outermost layer of the sample.

Measurements were recorded at 10-second intervals over a 2,000-second roasting period. Three biological replicates were conducted to ensure repeatability and reliability.

Predicted and Measured Temperature Comparisons

The CFD model provided temperature predictions at the same locations and time points as the experimental measurements. Table 2 summarises the predicted and experimentally measured temperatures (±*SD*) at 500, 1,000, 1,500, and 2,000 seconds.

Table 2. Validation of the Computational Model

Time [s]	Core temperature [°C]		Midpoint temperature [°C]		Surface temperature [°C]	
	CFD Prediction	Experimental	CFD Prediction	Experimental	CFD Prediction	Experimental
500	42.3 ±1.1	43.0 ±1.4	58.7 ±0.9	57.8 ±1.3	68.2 ±1.2	68.5 ±1.6
1,000	57.5 ±1.3	56.9 ±1.2	69.4 ±1.0	70.2 ±1.5	79.3 ±1.4	78.6 ±1.7
1,500	68.7 ±1.2	68.4 ±1.5	76.3 ±1.1	77.0 ±1.4	85.5 ±1.3	85.1 ±1.8
2,000	74.2 ±1.0	74.5 ±1.3	81.6 ±0.8	81.9 ±1.6	89.1 ±1.5	88.7 ±1.7

Statistical Validation

A Student's t-test was performed to evaluate the differences between the predicted and measured temperatures at each location and time point. The results showed no statistically significant differences ($P > 0.05$) between the predicted and experimental temperatures, confirming the model's accuracy. The maximum observed error was less than 2%, which is within an acceptable range for CFD applications in food processing. The validation results demonstrate that the CFD model provides highly accurate predictions of temperature distributions during thermal treatment. The alignment between the predicted and experimental temperatures at all measured points confirms the model's capability to capture the heat transfer dynamics and their impact on protein denaturation. The core temperature predictions exhibited minimal deviation, with a maximum difference of 0.9°C at 500 seconds. The surface temperature predictions consistently matched the experimental measurements within 0.5°C, indicating precise modelling of boundary heat transfer.

Stages of CFD Analysis

The CFD analysis was conducted in two phases, necessitated by the fluctuating output parameters during heat treatment and the model's size. The initial stage encompassed a steady-state analysis, yielding actual heat transfer coefficients on the surfaces of the meat sample and the GN container. In the subsequent phase, the outcomes from the first stage were used in modelling the solids (meat and GN container), and transient simulations were executed to capture the temperature variations of the samples over time. The initial phase focused on determining penetration coefficients, while the second phase involved simulating protein denaturation and water loss.

Roasting optimisation using RSM

In this experiment, the influence of three factors on the denaturation of myosin, collagen and actin, and mass loss was examined. The variables considered were the temperature within the furnace, air stream speed (fan speed), and humidity level. To interpret and characterise the relationship between these variables and the measured parameters, a quadratic equation was used. The central point was replicated six times in the model. Using Design-Expert version 11 (Stat-Ease, Inc., USA), the heat treatment was optimised concerning the denaturation of myosin, collagen and actin, and the cooking loss based on the model. The final step involved optimising the thermal treatment parameters using a mathematical model and experimental validation of the calculated response values.

Verification of the predicted results using laboratory tests

Materials

Turkey breast fillets (*Pectoralis major*) for analysis were obtained from a BIG 6 turkey acquired from a commercial market (INDYKPOL S.A., Olsztyn, Poland). The fillets were taken for further processing (1,954 ±174 g). The fillets were transported to the laboratory in cooling boxes, maintaining chilled conditions at 4±1°C. Cube-shaped samples (50 × 50 × 50 mm) were cut from turkey breasts according to Figure 2.

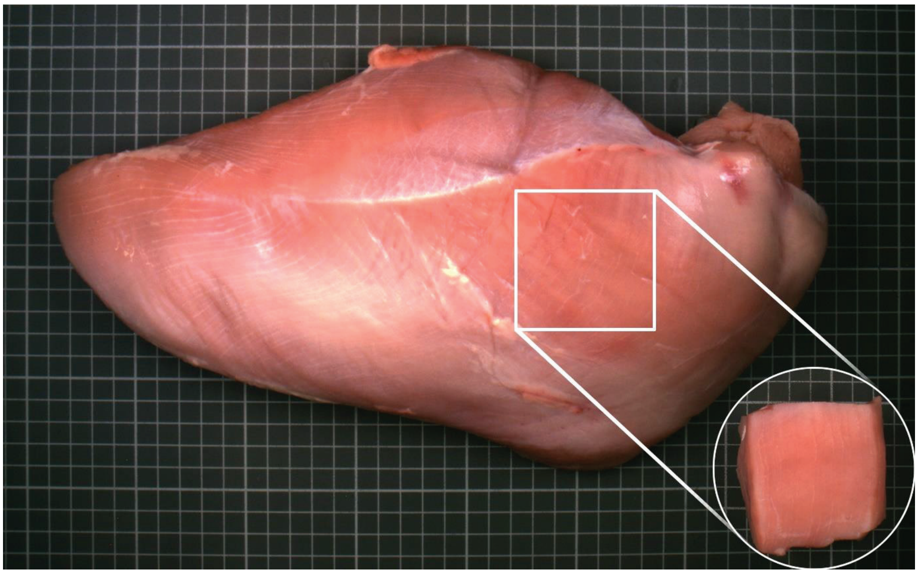

Figure 2. Preparation of turkey sample (50 × 50 × 50 mm) from breast fillets (Pectoralis major) for verification stage

Source: own elaboration.

Basic composition evaluation

The basic composition of turkey breast meat samples was evaluated using the near-infrared (NIR) spectrometry method as outlined by Stelmasiak et al. (2019) [22]. The analysis was conducted using a NIRFlex N-500 spectrometer with a solids module (Büchi Labortechnik AG, Switzerland) in reflectance mode, covering a spectral range of 12,500–4,000 cm^{-1}, and using a Büchi Art. N. N555-501. This analysis occurred at an accredited NIR laboratory (Polish Centre for Accreditation FT-NIR – Accreditation No. AB 1670). Homogenised meat samples, each weighing 100 g, were placed on a Petri dish and subjected to measurements. The assessment of the basic composition, encompassing water, fat, proteins, CTP (collagen protein total) and ash, was conducted in triplicate for each sample to ensure the precision and reliability of the results (three technical replications). Measurements were taken for each of the three independent biological replications (three different batches) involving distinct raw materials.

Heat treatment

The turkey meat cubes were thermally processed in accordance with the optimised parameters based on RSM data in a Küppersbusch CPE-110 combi-steam oven (Küppersbusch Convect-Air Professional, Germany). The process parameters were controlled using wireless temperature and humidity recorders (TrackSense Pro; Ellab, Denmark).

Protein denaturation level

Protein denaturation levels were assessed through differential scanning calorimetry (DSC 1 from Mettler Toledo, Schwerzenbach, Switzerland) [11]. Prior to the experiment, the device underwent calibration with pure zinc and indium. Both untreated and heat-treated samples were examined. A BÜCHI B-400 homogeniser was used for the uniform mixing of each sample. Subsequently, 10.0 ±0.1 mg of meat samples were deposited into a standard 40 µl aluminium pan (No.: ME-51119870) and hermetically sealed with an aluminium lid (No.: ME-51119871) using a Mettler Toledo Crucible Sealing Press.

Under an argon atmosphere (100 cm^3/min), the DSC analysis was conducted at a rate of 10°C/min (β) within the temperature range of 10°C to 100°C. The resultant thermograms were scrutinised using the STARe software to identify the initial (T_{on}), maximum (T_{max}), and final (T_{end}) temperatures, along with the enthalpy (ΔH). The degree of denaturation for myosin, collagen, and actin was ascertained based on the thermograms, using the methodology outlined by Agafonkina et al. (2019) [15]. The denaturation percentage for each protein was calculated by comparing the enthalpy of denaturation between the untreated and heat-treated samples, using equation (7).

$$\text{Denaturation \%} = \frac{H_{raw} - H_{roasted}}{H_{raw}} \times 100 \tag{7}$$

where:
H_{raw} – enthalpy of protein denaturation in raw meat prior to heat treatment (J/g),
$H_{roasted}$ – enthalpy of protein denaturation in roasted meat following heat treatment (J/g).

The evaluation of the degree of denaturation was conducted thrice for each sample to assure the precision and dependability of the outcomes (three technical replicates). Measurements were carried out across three independent biological replicates (three separate batches) using distinct raw materials for each.

Protein Denaturation Modelling Based on Temperature Predictions

To establish the connection between the temperature predictions from the Computational Fluid Dynamics (CFD) simulations and the extent of protein denaturation in the turkey breast meat, a detailed modelling approach was implemented. This section describes the methodology used to calculate the protein denaturation based on spatial and temporal temperature distributions.

Temperature Thresholds for Protein Denaturation

The Differential Scanning Calorimetry (DSC) data were used to identify the denaturation thresholds for the primary proteins in the turkey meat. The specific denaturation temperatures (±*SD*) and enthalpy values (ΔH) used in the model were as follows (Table 3):

Table 3. Denaturation thresholds and enthalpy changes (ΔH) for key turkey meat proteins

Protein	Denaturation Onset [T, °C]	Enthalpy Change [ΔH, J/g]
Myosin	61.5 ±0.8	3.85 ±0.12
Collagen	67.0 ±0.1	3.84 ±0.15
Actin	80.5 ±0.9	3.93 ±0.10

Source: own elaboration.

These thresholds represent critical points at which the respective proteins undergo irreversible structural changes, impacting the texture and functionality. The enthalpy data were integrated into kinetic equations to model the protein denaturation.

Spatial and Temporal Modelling of Protein Denaturation

1. Temperature Data from CFD Simulations:
   - The CFD model provided a three-dimensional grid of the turkey meat sample, with each grid element representing a localised region.
   - For each grid element, the transient temperature profile was extracted over the simulated roasting period (2,000 seconds).
2. Kinetic Model for Protein Denaturation:
   - Protein denaturation was modelled using a first-order kinetic equation:

$$\frac{d\alpha}{dt} = k(T)\cdot(1-\alpha)$$

   where:
   α(t) – degree of denaturation at time t,
   k(T) – temperature-dependent rate constant, calculated using the Arrhenius equation:

$$k(T) = A\cdot e^{\frac{-E_a}{RT}}$$

   with:
   A – pre-exponential factor [1/s],
   Ea – activation energy [J/mol],
   R – universal gas constant (8.314 J/mol·K),
   T – absolute temperature [K].
3. Spatial Variation in Denaturation:
   - Using the temperature data for each grid element, the degree of denaturation was calculated iteratively for each time step.
   - The enthalpy changes (ΔH) associated with each protein were incorporated into the calculations to adjust for localised thermal effects.
   - The resulting denaturation values were mapped across the 3D grid to visualise the spatial variation of protein denaturation within the meat sample.
4. Aggregation of Results:
   - The overall denaturation of each protein was determined by integrating the localised denaturation values across the entire sample. This approach ensured that spatial heterogeneity in heat transfer and protein response was accounted for.

Validation of Predicted Denaturation

Laboratory experiments were conducted to validate the predicted protein denaturation levels. Differential Scanning Calorimetry (DSC) was employed to measure the actual denaturation percentages for myosin, collagen, and actin in the samples subjected to the optimised roasting parameters. Table 4 compares the predicted and experimental results for protein denaturation and cooking loss:

Table 4. Comparison of CFD-predicted and experimentally measured protein denaturation and cooking loss

Parameter	Predicted value [±SD]	Experimental value [±SD]	P-value
Myosin Denaturation [%]	99.41 ±1.03	99.18 ±0.72	>0.05
Collagen denaturation [%]	88.37 ±1.02	87.67 ±0.75	>0.05
Actin denaturation [%]	20.70 ±0.78	19.21 ±0.32	>0.05

Source: own elaboration.

The predicted values from the CFD model showed strong agreement with the experimental results, with no statistically significant differences ($P > 0.05$) for all parameters. These results confirm the reliability of the model in predicting protein denaturation and cooking loss during thermal processing.

Cooking Loss

For the assessment of cooking loss, the sample underwent weighing before entering the oven and promptly after extraction. The percentage of cooking loss was computed by evaluating the disparity in mass between the raw and heat-treated meat using Equation (8).

$$\text{Cooking loss\%} = \frac{(M_{raw} - M_{roasted})}{M_{raw}} \times 100 \tag{8}$$

where:
M_{raw} – mass of the raw sample prior to heat treatment (g),
$M_{roasted}$ – mass of the roasted sample following heat treatment (g).

The evaluation of the cooking loss was conducted three times for each sample to guarantee the accuracy and reliability of the findings (three technical replicates). Measurements were carried out across three independent biological replicates (three distinct batches), using varied raw materials for each batch.

Statistics

The experimental design was executed using the Design-Expert v. 11 software (Stat-Ease, Inc., USA), as outlined in Table 1. An examination of the impact of temperature, humidity, and convection intensity on individual responses was conducted following the programmed experimental model. The significant terms within this model were identified through analysis of variance (ANOVA) for each response, assessing a lack of fit, coefficients of determination (R^2), and coefficients of variation (CV) to ensure model accuracy. 3D charts were constructed based on the analysis results, and quadratic equations describing the model were used for further studies.

In the prediction analysis using RSM, the maximum desirable degree of myosin and collagen denaturation was determined, with the minimisation of actin denaturation and mass loss selected as parameters.

The final stage of the experiment involved optimising and validating the heat treatment technology. Predicted response values were then compared with experimentally determined values. Instrumental technique analyses were conducted using raw materials from various production batches in three independent biological replicates. Following optimisation, Student's *t*-test at $P \leq 0.05$ was applied to ascertain differences between two sets of values: predicted and measured properties in the laboratory experiments. Statistical analyses were performed using the Design-Expert software, and the results were presented as mean ($\bar{X}$) ± standard error (SD).

RESULTS AND DISCUSSION

Table 5 summarises the regression coefficients of the quadratic polynomial models predicting myosin, collagen and actin denaturation, and cooking loss. The models exhibited high adequacy (R^2 = 0.746–0.997). Myosin, collagen, and actin denaturation achieved the highest R^2 values (0.933, 0.984, 0.997), while cooking loss was the least predictive (R^2 = 0.746). Finding all lack-of-fit p-values confirmed satisfactory model fits (p >0.05).

Table 5. Regression coefficients of the predicted quadratic polynomial models for the physical values of the of myosin, collagen and actin denaturation, and cooking loss.

Factor	Denatured myosin [%]	Denatured collagen [%]	Denatured actin [%]	Cooking loss [%]
Intercept	96.27	86.39	17.76	20.32
Temp	2.71***	17.00***	24.01***	2.96**
Hum	2.68***	3.71***	6.20***	0.26
Fan	1.12*	1.71*	4.12***	2.18*
Temp × Hum	−2.56***	−1.40	2.66*	−0.10
Temp × Fan	−0.66	−1.00	3.31***	−0.75
Hum × Fan	−0.56	−0.23	−2.76*	−0.10
$Temp^2$	−0.38	−7.75***	26.44***	0.67
Hum^2	−0.63	0.40	−1.31	0.37
Fan^2	0.56	1.00	0.89	−0.43
R^2	0.933	0.984	0.997	0.746
Lack of fit	0.261	0.289	0.281	0.081

Temp – Temperature, Hum – Humidity, Fan – Fan rotation speed, R^2 – square coefficient of the fitting model, Lack of fit – p-value of lack of fit.
* – Significant at $P \leq 0.05$; ** – Significant at $P \leq 0.01$; *** – Significant at $P \leq 0.001$.

Source: own elaboration.

Optimisation of heat treatment parameters

RSM was used to assess the influence of temperature, humidity, and fan speed on the heat treatment of turkey breast fillets (*Pectoralis major*). This modelling technique made it possible to observe trends in the outcomes as the independent variables were altered. The regression coefficients outlined in Table 5, along with the 3D plots depicted in Figures 3 and 4, demonstrated that all three independent variables exerted a statistically significant effect on the denaturation levels of individual proteins, encompassing myosin, collagen, actin, and cooking loss in the meat samples. However, the extent of this impact varied depending on the specific response and the level of the independent variable.

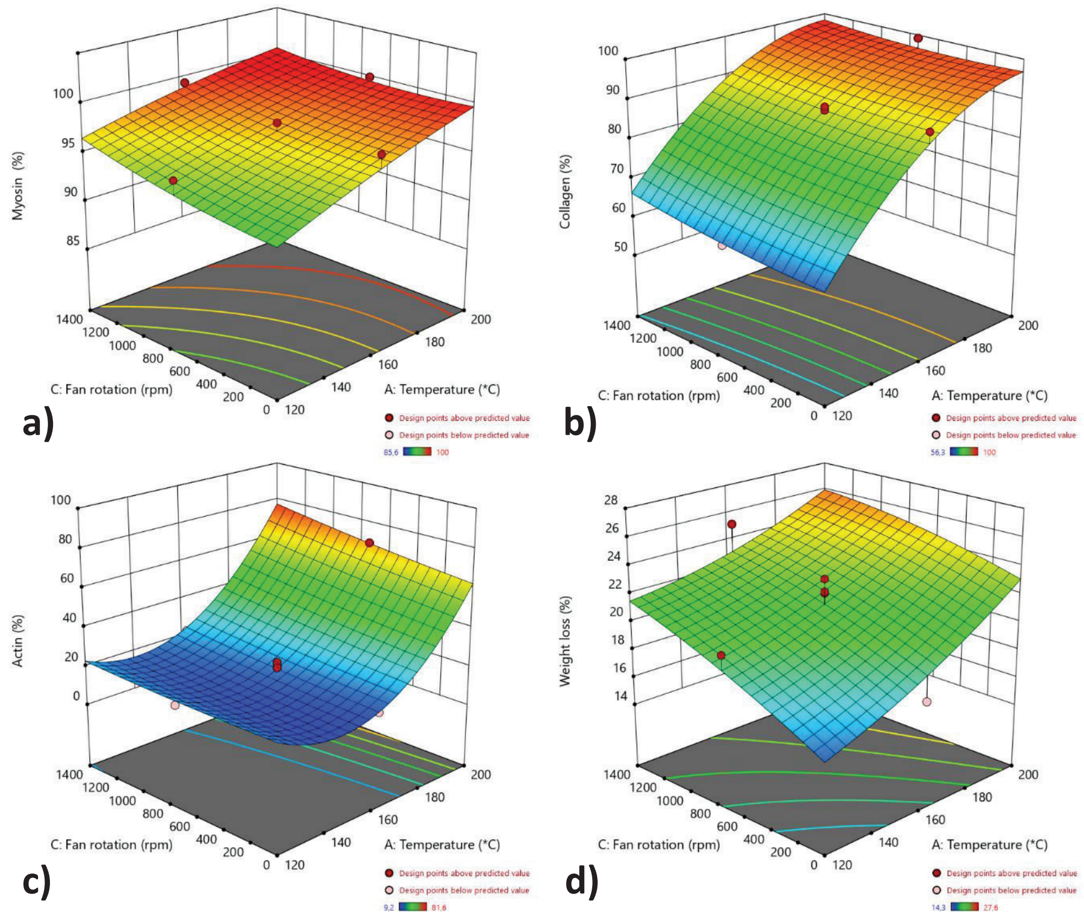

Figure 3. 3D surface charts generated using the RSM model at a humidity of 37.5% illustrate the impact of factors and their interactions on output data: a – myoglobin denaturation [%], b – collagen denaturation [%], c – actin denaturation [%], and d – cooking loss [%]

Source: own elaboration.

The degree of myosin denaturation was significantly influenced by temperature and humidity in linear terms ($P \leq 0.001$), with a similar linear effect observed for fan rotation speed ($P \leq 0.05$). However, quadratic effects of these parameters were not significant ($P > 0.05$), nor were the interactions involving fan speed with temperature or humidity ($P > 0.05$). The strong linear relationship between temperature and myosin denaturation aligns with previous studies, indicating that muscle proteins, including myosin, are highly sensitive to heat. High temperatures disrupt hydrogen and hydrophobic bonds, leading to structural changes that impact protein functionality and digestibility [23]. Similarly, the role of humidity in accelerating denaturation highlights the need for precise humidity control during thermal processing to maintain optimal texture and nutritional quality [24]. The observed linear effect of fan speed suggests that enhanced air circulation facilitates heat transfer and protein denaturation by promoting water evaporation from the meat's surface [25]. The significant interaction between temperature and humidity ($P \leq 0.001$) underscores the necessity of balancing these factors, as low humidity combined with high temperatures can adversely affect meat quality [26]. These findings confirm that the primary factors influencing myosin denaturation operate in a linear fashion, with a limited impact of more complex interactions.

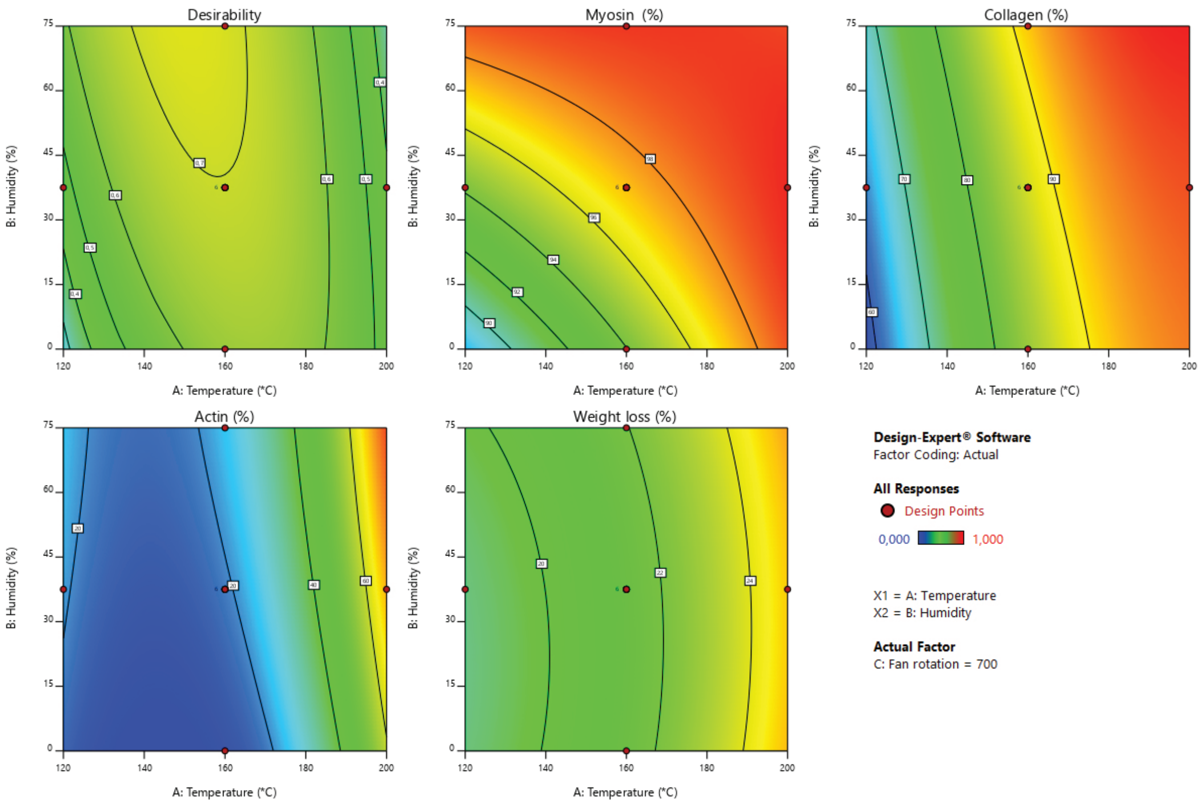

Figure 4. Desirability plots and contour graphic solutions of RSM optimisation

Source: own elaboration.

The investigation into collagen denaturation in turkey breast fillets revealed that temperature, humidity, and fan rotation speed significantly influenced collagen denaturation in linear terms ($P \leq 0.001$, $P \leq 0.001$, $P \leq 0.05$, respectively). Among these, temperature emerged as the most critical factor, with both significant linear and quadratic effects ($P \leq 0.001$). Conversely, humidity and air movement speed did not exhibit statistically significant quadratic effects ($P > 0.05$). Interaction effects between temperature × fan rotation speed, temperature × humidity, and humidity × fan rotation speed were also not statistically significant ($P > 0.05$). These findings align with previous studies on myosin denaturation and other muscle proteins, emphasising temperature as the primary driver of protein denaturation. For instance, computational fluid dynamics (CFD) simulations in pork and beef confirm temperature's dominant role, while humidity and airflow typically play secondary roles [10, 15]. The quadratic effect of temperature reflects the non-linear progression of denaturation at higher temperatures, where collagen breakdown becomes more pronounced. Similar trends have been reported in studies on other meats, such as Atlantic salmon, where environmental factors like air circulation showed limited interactive effects compared to temperature [18]. The results underscore the importance of precise temperature control during meat processing, especially for products intended for children and adolescents, where maintaining protein integrity and nutrient retention is critical for a healthy diet.

All the analysed factors – temperature, humidity, and fan rotation speed – exhibited statistically significant effects on actin denaturation in linear terms ($P \leq 0.001$). Interactions among these factors, such as temperature × × humidity and temperature × fan rotation speed, also had significant impacts ($P \leq 0.05$, $P \leq 0.001$, respectively). However, humidity and fan speed did not show significant quadratic effects ($P > 0.05$), whereas temperature did ($P \leq 0.001$). Temperature emerged as the most critical parameter, influencing actin denaturation both linearly and quadratically ($P \leq 0.001$). Actin, a heat-sensitive protein, begins denaturing around 66°C, with further increases in temperature accelerating the process, as confirmed by CFD simulations and studies on other meats. Elevated temperatures drive moisture loss and textural changes, aligning with findings on pork loin and other muscle foods [10]. Humidity significantly impacted actin denaturation in linear terms, likely due to its role in moisture retention within the meat matrix. Although its quadratic effects were negligible, maintaining optimal humidity is essential to balance moisture retention with heat for effective denaturation. Fan speed influenced denaturation indirectly, primarily through enhanced heat distribution, as shown in the CFD models. However, its effects plateau beyond certain levels, reflecting findings from similar studies [27, 28]. In summary, temperature dominates the dynamics of actin denaturation in turkey meat, with humidity and fan speed playing secondary but complementary roles, particularly in linear interactions.

The study investigated how temperature, fan speed, and humidity influence cooking loss during the roasting of turkey breast fillets. Temperature and fan speed significantly affected cooking loss in a linear manner ($P \leq 0.01$ and $P \leq 0.05$, respectively), while chamber humidity showed no significant effect ($P > 0.05$). No significant interaction effects between temperature, fan speed, and humidity were observed, nor were there significant quadratic effects for any factor ($P > 0.05$). Temperature emerged as the dominant factor influencing cooking loss, aligning with established research indicating that higher temperatures expedite protein denaturation and collagen shrinkage, leading to increased water release and weight loss [29]. Similarly, fan speed's significant but smaller effect was attributed to enhanced surface evaporation, which is consistent with studies demonstrating the role of air velocity in drying rates [30]. Interestingly, the lack of a significant impact of humidity ($P > 0.05$) on cooking loss is contrary to findings in certain studies, where high humidity environments during roasting have been reported to slow down moisture evaporation and reduce overall weight loss [31]. Contrary to some findings, humidity did not mitigate weight loss under the tested conditions, possibly due to the specific temperature and fan speed settings. These results reinforce the importance of temperature and air circulation in optimising roasting conditions to minimise cooking loss while maintaining product quality. Prior studies, such as those by Bıyıklı et al. and Gál et al. [23, 32], corroborate that processing temperature and time influence the weight loss, texture, and sensory properties. For example, higher temperatures paired with shorter processing times were associated with lower weight losses and improved sensory qualities, highlighting the interplay between the thermal conditions and meat characteristics. These findings provide valuable insights for designing healthier cooking methods tailored for children and adolescents [23, 32].

VERIFICATION OF HEAT TREATMENT PARAMETERS

The final step of the experiment involved validating the results of the RSM model by comparing them with laboratory tests. The maximal desirable denaturation of myosin and collagen was determined, while actin denaturation and mass loss were minimised. According to the RSM optimisation model, the ideal processing conditions for turkey breast meat are a temperature of 161.28°C, humidity of 61.31%, and a fan speed set at 17.58 rpm. The predicted values for the output parameters ($\bar{X}$ ±SD) were as follows: myosin denaturation at 99.41 ±1.03%, collagen denaturation at 88.37 ±1.02%, actin denaturation at 20.70 ±0.78%, and cooking loss at 18.93 ±0.13%. The results of the optimisation and verification process are presented in Table 6, indicating that the laboratory test outcomes were consistent with the predictions of the RSM model. There were no statistically significant differences observed between the predicted and laboratory test values.

Table 6. Verification of the RSM model with laboratory tests ($\bar{X}$ ±*SD*)

Design factors	Optimum heat treatment parameters	
Temperature [°C]	161.28	
Humidity [%]	61.31	
Fan rotation [RPM]	17.58	
Responses	Predicted values	Laboratory tests values
Myosin denaturation [%]	99.41 ±1.03	99.18 ±0.72
Collagen denaturation [%]	88.37 ±1.02	87.67 ±0.75
Actin denaturation [%]	20.70 ±0.78	19.21 ±0.32
Weight loss [%]	18.93 ±0.13	16.24 ±0.15

*Letters (A, B) show the significant differences between predicted and laboratory tests values ($P \leq 0.05$).

Source: own elaboration.

CONCLUSIONS AND FUTURE PERSPECTIVES

The work presents the innovative use of the CFD method to predict protein denaturation in turkey breast meat and optimise heat treatment processes. Using mathematical models, the optimal thermal treatment conditions were determined, which were 161.28°C, 61.31% air humidity, and the fan speed set at 17.58 rpm. The denaturation of various proteins and losses during the baking process was assessed. During the verification of the laboratory results, it was found that the denaturation of myosin and actin, as well as the losses during the cooking process, did not differ significantly from the values predicted on the basis of the response surface model developed based on simulation data. The study proves that the CFD method can be a valuable tool for predicting protein denaturation and losses in the cooking process of turkey breast meat, which can improve the quality and efficiency of products in the food industry.

Acknowledgements
Research financed by the Polish Ministry of Science and Higher Education within funds of Faculty of Human Nutrition and Consumer Sciences, Warsaw University of Life Sciences (WULS), for scientific research.

REFERENCES

[1] **Chauhan A., Kaur H., Yadav S., Jakhar S.K. 2020.** A Hybrid Model for Investigating and Selecting a Sustainable Supply Chain for Agri-Produce in India. Annals of Operations Research 290: 621–642. https://www.doi.org/10.1007/s10479-019-03190-6

[2] **Liu Y., Wang Z., Zhang D., Pan T., Liu H., Shen Q., Hui T. 2022.** Effect of Protein Thermal Denaturation on the Texture Profile Evolution of Beijing Roast Duck. Foods 11: 1–14. https://www.doi.org/10.3390/foods11050664

[3] **Khalid W., Maggiolino A., Kour J., Arshad M.S., Aslam N., Afzal M.F., Meghwar P., Zafar K.U.W., De Palo P., Korma S.A. 2023.** Dynamic Alterations in Protein, Sensory, Chemical, and Oxidative Properties Occurring in Meat during Thermal and Non-Thermal Processing Techniques: A Comprehensive Review. Frontiers in Nutrition 9: 1–19. https://www.doi.org/10.3389/fnut.2022.1057457

[4] **Szpicer A., Bińkowska W., Stelmasiak A., Zalewska M., Wojtasik-Kalinowska I., Piwowarski K., Półtorak A. 2024.** Innovative Implementation of Computational Fluid Dynamics in Proteins Denaturation Process Prediction in Goose Breast Meat and Heat Treatment Processes Optimization. Applied Sciences 14: 1–22. https://www.doi.org/0.3390/app14135567

[5] **De Albuquerque C.D., Curet S., Boillereaux L. 2019.** A 3D-CFD-Heat-Transfer-Based Model for the Microbial Inactivation of Pasteurized Food Products. Innovative Food Science & Emerging Technologies 54: 172–181. https://www.doi.org/10.1016/j.ifset.2019.04.007

[6] **Norton T., Sun D.W. 2006.** Computational Fluid Dynamics (CFD) – an Effective and Efficient Design and Analysis Tool for the Food Industry: A Review. Trends in Food Science & Technology 17: 600–620. https://www.doi.org/10.1016/j.tifs.2006.05.004

[7] **James C., Vincent C., de Andrade Lima T.I., James S.J. 2006.** The Primary Chilling of Poultry Carcasses. A Review. International Journal of Refrigeration 29: 847–862. https://www.doi.org/10.1016/j.ijrefrig.2005.08.003

[8] **Park H.W., Yoon W.B. 2018.** Computational Fluid Dynamics (CFD) Modelling and Application for Sterilization of Foods: A Review. Processes 6: 1–14. https://www.doi.org/10.3390/pr6060062

[9] **Szpicer A., Binkowska W., Stelmasiak A., Wojtasik-Kalinowska I., Wierzbicka A., Poltorak A. 2023.** Application of Computational Fluid Dynamics Simulation in Predicting Food Protein Denaturation : Numerical Studies on Selected Food Products. A Review. Animal Science Papers and Reports 41: 307–332. https://www.doi.org/10.2478/aspr-2023-0014.

[10] **Szpicer A., Binkowska W., Wojtasik-Kalinowska I., Półtorak A. 2023.** Prediction of Protein Denaturation and Weight Loss in Pork Loin (Muscle Longissimus Dorsi) Using Computational Fluid Dynamics. European Food Research and Technology 249: 3055–3068. https://www.doi.org/10.1007/s00217-023-04348-0

[11] **Szpicer A., Wierzbicka A., Półtorak A. 2022.** Optimization of Beef Heat Treatment Using CFD Simulation: Modeling of Protein Denaturation Degree. Journal of Food Process Engineering 45: e14014. https://www.doi.org/10.1111/jfpe.14014

[12] **Zielbauer B.I., Franz J., Viezens B., Vilgis T.A. 2016.** Physical Aspects of Meat Cooking: Time Dependent Thermal Protein Denaturation and Water Loss. Food Biophysics 11: 34–42. https://www.doi.org/10.1007/s11483-015-9410-7

[13] **Hassoun A., Aït-Kaddour A., Sahar A., Cozzolino D. 2021.** Monitoring Thermal Treatments Applied to Meat Using Traditional Methods and Spectroscopic Techniques: A Review of Advances over the Last Decade. Food and Bioprocess Technology 14: 195–208. https://www.doi.org/10.1007/s11947-020-02510-0

[14] **Latorre M.E., Palacio M.I., Velázquez D.E., Purslow P.P. 2019.** Specific Effects on Strength and Heat Stability of Intramuscular Connective Tissue during Long Time Low Temperature Cooking. Meat Science 153: 109–116. https://www.doi.org/10.1016/j.meatsci.2019.03.016.

[15] **Agafonkina I.V., Korolev I.A., Sarantsev T.A. 2019.** The Study of Thermal Denaturation of Beef, Pork, Chicken and Turkey Muscle Proteins Using Differential Scanning Calorimetry. Theory and Practice of Meat Processing 4: 19–23. https://www.doi.org/10.21323/2414-438x-2019-4-3-19-23.

[16] **Choi Y., Okos M.R. 1986.** Thermal Properties of Liquid Foods – Review. American Society of Association Executives. 35–77.

[17] **Gantner M., Guzek D., Najda A., Brodowska M., Górska-Horczyczak E., Wojtasik-Kalinowska, I., Godziszewska J. 2017.** Oxidative and Microbial Stability of Poultry Meatballs Added with Coriander Extracts and Packed in Cold Modified Atmosphere. International Journal of Food Properties 20: 2527–2537. https://www.doi.org/10.1080/10942912.2016.1243125

[18] **Szpicer A., Binkowska W., Wojtasik-Kalinowska I., Stelmasiak A., Poltorak A. 2024.** Hybrid Method for Predicting Protein Denaturation and Docosahexaenoic Acid Decomposition in Atlantic Salmon (*Salmo Salar* L.) Using Computational Fluid Dynamics and Response Surface Methodology. European Food Research and Technology 250, 4: 1–14. https://www.doi.org/10.1007/s00217-023-04453-0

[19] **Srikiatden J., Roberts J.S. 2007.** Moisture Transfer in Solid Food Materials: A Review of Mechanisms, Models, and Measurements. International Journal of Food Properties 10: 739–777. https://www.doi.org/10.1080/10942910601161672

[20] **Singh R.P., Heldman D.R. 2014.** Introduction to Food Engineering: Fifth Edition. Elsevier, London.

[21] **Markočič E., Knez Ž. 2016.** Redlich-Kwong Equation of State for Modelling the Solubility of Methane in Water over a Wide Range of Pressures and Temperatures. Fluid Phase Equilibria 408 108–114. https://www.doi.org/10.1016/j.fluid.2015.08.021

[22] **Stelmasiak A., Wyrwisz J., Wierzbicka A. 2019.** Effect of Packaging Methods on Salt-Reduced Smoked-Steamed Ham Using Herbal Extracts. CyTA – Journal of Food 17: 834–840. https://www.doi.org/10.1080/19476337.2019.1660409

[23] **Gál R., Kameník J., Salek R.N., Polášek Z., Macharáčková B., Valenta T., Haruštiaková D., Vinter Š. 2022.** Research Note: Impact of Applied Thermal Treatment on Textural, and Sensory Properties and Cooking Loss of Selected Chicken and Turkey Cuts as Affected by Cooking Technique. Poultry Science 101: 1–6. https://www.doi.org/10.1016/j.psj.2022.101923

[24] **Zhang Y., Dong L., Zhang J., Shi J., Wang Y., Wang S. 2021.** Adverse Effects of Thermal Food Processing on the Structural, Nutritional, and Biological Properties of Proteins. Annual Review of Food Science and Technology 12: 259–286. https://www.doi.org/10.1146/annurev-food-062320-012215

[25] **Desmond E. 2006.** Reducing Salt: A Challenge for the Meat Industry. Meat Science 74: 188–196. https://www.doi.org/10.1016/j.meatsci.2006.04.014

[26] **Kim Y.H.B., Warner R.D., Rosenvold K. 2014.** Influence of High Pre-Rigor Temperature and Fast PH Fall on Muscle Proteins and Meat Quality: A Review. Animal Production Science 54: 375–395. https://www.doi.org/10.1071/AN13329

[27] **Abraha B., Admassu H., Mahmud A., Tsighe N., Shui X.W., Fang Y. 2018.** Effect of Processing Methods on Nutritional and Physico-Chemical Composition of Fish: A Review. MOJ Food Processing & Technology 6: 376–382. https://www.doi.org/10.15406/mojfpt.2018.06.00191

[28] **Yesiltas B., García-Moreno P.J., Sørensen A.D.M., Akoh C.C., Jacobsen C. 2019.** Physical and Oxidative Stability of High Fat Fish Oil-in-Water Emulsions Stabilized with Sodium Caseinate and Phosphatidylcholine as Emulsifiers. Food Chemistry 276: 110–118. https://www.doi.org/10.1016/j.foodchem.2018.09.172

[29] **Tornberg E. 2005.** Effects of Heat on Meat Proteins – Implications on Structure and Quality of Meat Products. Meat Scince 70: 493–508. https://www.doi.org/10.1016/j.meatsci.2004.11.021

[30] **Murphy R.Y., Johnson E.R., Duncan L.K., Clausen E.C., Davis M.D., March J.A. 2001.** Heat Transfer Properties, Moisture Loss, Product Yield, and Soluble Proteins in Chicken Breast Patties during Air Convection Cooking. Poultry Science 80: 508–514. https://www.doi.org/10.1093/ps/80.4.508

[31] **Suleman R., Wang Z., Aadil R.M., Hui T., Hopkins D.L., Zhang D. 2020.** Effect of Cooking on the Nutritive Quality, Sensory Properties and Safety of Lamb Meat: Current Challenges and Future Prospects. Meat Science 167: 108172. https://www.doi.org/10.1016/j.meatsci.2020.108172

[32] **Bıyıklı M., Akoğlu A., Kurhan Ş., Akoğlu İ.T. 2020.** Effect of Different Sous Vide Cooking Temperature-Time Combinations on the Physicochemical, Microbiological, and Sensory Properties of Turkey Cutlet. International Journal of Gastronomy and Food Science 20: 100204. https://www.doi.org/10.1016/j.ijgfs.2020.100204

DOI: 10.22630/TPFP.2024.1.10032

ORIGINAL PAPER
Received: 15.10.2024
Accepted: 20.12.2024

ORGANIC FOOD FROM THE PERSPECTIVE OF PARENTS OF CHILDREN WITH AUTISM SPECTRUM DISORDERS

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Abstract: The number of children diagnosed with autism spectrum disorder (ASD) is increasing worldwide. Children with ASD often have impaired detoxification capacity, gastrointestinal problems and food intolerances. A well-balanced diet based on organic foods can play a significant role in alleviating both metabolic and psychological symptoms. The aim of this study was to explore the opinions and attitudes of parents of children with ASD towards organic foods. The study was conducted between June 2021 and May 2022 using a survey method, among 96 respondents. Those who were more knowledgeable about proper nutrition (19% of respondents) and those who used special diets for their children (45%) were more likely than the other respondents to believe that organic foods could improve the functioning of children with ASD. These parents were also characterised by better knowledge of organic food (definition, labelling). The most important factors when choosing organic food were health considerations, chemical content and simple product composition. The main source of information about organic food was the Internet (88%) and the most common place to buy was a specialist shop (43%). Parents who purchased organic food most frequently chose vegetables and fruit (69%) and eggs (65%). The main reasons respondents gave for not purchasing organic food were the high price and a lack of trust towards producers and certification bodies. In light of the collected data, it seems justified to take actions aimed at raising parents' knowledge of proper diet and nutrition, which could result in increased consumer awareness of organic food.

Key words: organic food, autism, diets, nutrition, knowledge, consumer preferences

INTRODUCTION

The number of children diagnosed with autism spectrum disorder (ASD) is increasing worldwide [1]. The cause of these disorders has not yet been established. The etiology is believed to be multifactorial, involving genetic predisposition, environmental factors, as well as factors related to immune system response and gastrointestinal function [2]. Children with ASD have impaired detoxification capacity and are particularly

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vulnerable to chemical contaminants from the environment, food, water and air. Correlations have been observed between the severity of autistic symptoms and the presence of heavy metals in the bodies of children with autism, as well as glutathione deficiency, which hinders detoxification processes [3–5]. A diet based on raw and organic products should be recommended for this group of patients. Organic food of plant origin is usually less frequently contaminated with pesticide residues when compared to the conventionally cultivated crops. Controls conducted in the European Union in 2018 showed that 44.5% of conventional food samples contained residues of one or more pesticides, and 1.2% of samples exceeded the maximum pesticide residue limits for more than 100 pesticides. In the case of organic products, 6.5% of the samples analysed contained residues of one or more pesticides, and only 3 samples (0.2%) exceeded the maximum residue limits for 3 pesticides [6].

Children with ASD are more likely than their neurotypical peers to experience gastrointestinal problems and abnormal eating behaviours. Sensory disorders, as well as the use of elimination diets, can contribute to a poorer diet, leading to insufficient intake of nutrients and their deficiencies [7]. Many studies indicate that children with ASD consume less than the recommended daily intake of calcium, iron, zinc, and vitamins A, E, K, D, C, B_6, B_{12}, and folic acid [8,9]. The intake of vitamins and compounds with antioxidant properties may be important due to coexisting metabolic disorders in children with ASD, including elevated blood levels of oxidative stress markers, which can affect the functioning of the central nervous system [10]. Numerous studies comparing the content of minerals and biologically active substances in plants from organic versus conventional farming indicate that organic food contains higher levels of certain nutrients, such as iron, magnesium, phosphorus, potassium, calcium, vitamin C, and polyphenols [11–13]. A diet enriched with calcium is particularly important for children with autism on a dairy-free diet, as this group has been observed to have lower bone mineral density and lower blood vitamin D levels than neurotypical children of the same age [14]. Milk produced in the organic system, compared to milk from conventionally raised cows, has a higher nutritional value: it contains more dry matter, fat, calcium, as well as vitamin C and α-tocopherols [13].

Children with autism are at risk of deficiencies in n-3 polyunsaturated fatty acids, which may lead to developmental disorders of the central nervous system, contributing to dyslexia, dyspraxia, hyperactivity, and reduced concentration. Studies on the effects of omega-3 supplementation indicate overall health improvements in children with ASD, including better sleep patterns, enhanced cognitive and motor skills, improved concentration, eye contact, social behaviours, as well as a reduction in stereotypies, anxiety, aggression, and hyperactivity [15]. Consuming organic food could potentially help improve these areas of functioning in this group of children. Organic milk and dairy products contain more health-promoting omega-3 unsaturated fatty acids and a better ratio of these acids to omega-6. Similarly, organic meat products, compared to conventionally raised meat, contain higher concentrations of polyunsaturated fatty acids (PUFAs), especially n-3 PUFAs [16, 17].

The results obtained so far indicate that children with ASD are more at risk of obesity than their healthy peers, and that the prevalence of overweight and obesity is higher in children with autism [18]. Human studies suggest that regular and frequent consumption of organic food may help reduce the incidence of conditions such as overweight and obesity, as well as allergic and cancerous diseases [13]. A well-balanced diet based on nutrient-rich, contaminant-free food can play a significant role in treating and alleviating digestive-metabolic and psychological symptoms in children with ASD.

Parents of children with autism are often actively engaged in seeking knowledge about a healthy lifestyle and alternative methods to support their children's development. The organic food market can be a place for them to fulfil their pro-health and pro-environmental values. Understanding their motivations will help tailor marketing and educational efforts to the specific needs of this group of consumers.

The aim of the study was to explore the opinions and knowledge of parents of children with autism spectrum disorders (ASD) regarding organic food. The study also examined their consumer preferences concerning the purchase and consumption of organic food products.

MATERIALS AND METHODS

The study was conducted between June 2021 and May 2022. It received approval from the Ethics Committee for Scientific Research Involving Humans at the Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (SGGW), under Resolution No. 20/2021. The CAWI survey method was used with a custom questionnaire consisting of 32 questions concerning, among others things, the general characteristics of the respondents, the use of an elimination diet for their child/children with ASD, knowledge and opinions about organic food, as well as the motives, frequency, and places of purchasing organic products. The question regarding respondents' nutritional knowledge included elements from the KomPAN questionnaire for assessing dietary beliefs and habits [19]. The questionnaire included single- and multiple-choice closed questions, closed questions with an open-response option, and open-ended questions. To assess respondents' opinions on the factors influencing their decisions to purchase organic food, a 5-point Likert scale was used, where 5 indicated 'very important' and 1 'not important'. Respondents also had the option to choose 'no opinion'. The study targeted parents of underage children diagnosed with autism spectrum disorders (ASD) living in Poland. The sample was selected using the snowball method and voluntary sampling. The invitation to participate in the study was shared via email, social media, and online platforms focused on parents of children with ASD.

In the analysis of the results, the percentage share of individual responses was presented, and to determine the statistical significance of the relationship between the variables, Pearson's correlation coefficient was calculated using the STATISTICA program.

A total of 96 respondents participated in the study (Table 1), of which more than 87% were women. The majority were between the ages of 30 and 50 (91%). Responses were collected from residents of all Polish provinces, but the largest groups were from the Mazowieckie province (38.5% of respondents) and the Małopolskie province (27.1%). 69% of respondents lived in large or medium-sized cities, while over 18% lived in rural areas. The largest group of respondents had a university degree (80.2% of those surveyed). The highest percentage of respondents (about 20%) reported an average monthly income per household member in the range of PLN 1001–2500, while the lowest percentage (about 3%) declared an income of PLN 1000 or less. More than 36% of respondents did not wish to disclose income information. Considering the subjective assessment of material conditions, over half of the 96 respondents stated that they were very satisfied or satisfied (8.3% and 44.8%, respectively) with their financial situation. About one-third (34.4%) gave a neutral response, saying they were neither satisfied nor dissatisfied, while 12.5% expressed dissatisfaction with their financial situation. None of the respondents described their financial situation as 'very dissatisfied'.

According to previous studies [20–23], this type of consumer should be interested in organic food. This type of food is primarily purchased by residents of large cities who are high-school or university educated and consider their financial situation good.

Table 1. Participant characteristics

Socio-economic characteristic		Number of respondents (n = 96)	%
Gender	women	83	87.4
	men	13	12.6
Age (years)	18–30	1	1
	31–40	56	58.3
	41–50	35	36.5
	above 50	4	4.2
Place of residence	village	18	18.8
	town up to 40,000 inhabitants	11	11.5
	city up to 100,000 inhabitants	12	12.5
	city over 100,000 inhabitants	55	57.3
Education	primary	1	1
	vocational	4	4.2
	high school	14	14.6
	university	77	80.2
Household net income (PLN) per person/month	1,000 or less	3	3.1
	1,001–2,500	20	20.8
	2,501–3,500	10	10.4
	3,501–5,000	13	13.5
	above 5,000	15	15.6
	no declaration	35	36.5

Source: own elaboration.

RESULTS AND DISCUSSION

It is assumed that individuals with a higher level of education possess greater knowledge about proper nutrition and are more inclined to purchase organic food. The questionnaire included a request for self-assessment of one's knowledge in the area of proper diet and nutrition. The largest group consisted of parents who rated their nutritional knowledge as sufficient (44 responses – 45.8% of respondents). Nearly one-third of the surveyed individuals believed that their nutritional knowledge was at a good level, while over 18% gave themselves a failing grade. Only 5% of parents rated their knowledge in this area as very good.

After analysing the respondents' answers to the questions concerning selected issues of proper nutrition, it was found that over 36% of the respondents rated their level of knowledge accurately, nearly 47% overestimated their knowledge, and just over 16% assessed their knowledge as being lower than the actual level. It should be emphasised that no significant correlation was found between self-assessed knowledge of proper diet and nutrition and the knowledge assessment assigned to respondents based on their answers to the questions in this area ($r = 0.125$).

Since, as mentioned, the cause of autism has not been diagnosed, standards of treatment for this group of patients have not yet been established. Parents of children with autism spectrum disorders (ASD), facing their children's developmental challenges, include various forms of therapy in their therapeutic efforts. Several studies have shown that younger children are more likely to receive dietary interventions (restrictive diets and dietary supplements) as well as educational and behavioural interventions. Pharmacological interventions are more widely used among adolescents [24]. The most commonly used dietary intervention is the gluten-free and casein-free diet (GFCF) [25]. The study found that some parents (43 individuals – 44.8% of the respondents) also made changes to their child's and family members' diets (Table 2). The most frequently implemented diets were gluten-free (GF), dairy-free (casein-free CF), and those restricting simple sugars and sucrose (sugar-free – SF).

Table 2. Types of elimination diets introduced by respondents in children with ASD

Type of diet	Children with ASD ($n = 43$)
elimination of allergens and intolerance	4
dairy-free (CF)	5
sugar-free (SF)	1
gluten-free (GF)	2
dairy-free (CF) + sugar-free (SF)	4
gluten-free (GF) + dairy-free (CF)	3
gluten-free (GF) + dairy-free (CF) + sugar-free (SF)	17
gluten-free (GF) + dairy-free (CF) + sugar-free (SF) + other: low FODMAP (3), soy- and starch-free (1)	4
vegetarian	2
ketogenic	1

Source: own elaboration.

It was assumed that individuals introducing an elimination diet in their families would seek to educate themselves on proper nutrition and thus have greater knowledge of organic food. Research conducted among Polish consumers [20, 26] indicated that factors such as interest in diets and proper nutrition influenced a greater openness to organic products. For this reason, the understanding of issues related to organic food (definition, labelling of organic products) was assessed, taking into account the respondents' level of knowledge about proper diet and nutrition, as well as their use of special diets (Table 3).

The study found that the majority of respondents (77 responses – 80.2%) recognised the correct definition of organic food. However, as many as 62.5% of respondents (multiple answers were allowed) indicated that organic food is 'food produced without synthetic fertilisers and pesticides'. This is an incorrect answer, even though it refers to production methods in organic farming. Most parents knew which terms on a product guaranteed that it came from organic farming. The most recognised label was EKO (65.6% of responses), while the least recognised was 'organic' (19.8% of selected responses). Some respondents confused the concept of organic food with healthy food (16.7% of responses) or preservative-free food (11.5% of responses). This may result from insufficient consumer knowledge and/or unfair practices of traders that misuse terms associated with organic food. Knech and Gurwin [21] also note the issue of inconsistency in the messages delivered to customers. The consequence of such actions is poor product identification and, subsequently, the inability to distinguish organic food from conventional food.

Table 3. Structure of respondents' answers based on their level of knowledge about proper nutrition and the use of a special diet for a child with ASD

Question	Structure of responses regarding knowledge and opinions about organic food [%]				
	assessment of the level of knowledge about proper nutrition			diet for a child with ASD	
	insufficient *n* = 42	adequate *n* = 34	good *n* = 18	yes *n* = 43	no *n* = 53
Definition of organic food*					
Any natural, unprocessed food	31	19.4	16.7	18.06	28.30
Food produced by organic farming methods	**66.7**	**86.1**	**94.4**	**86.05**	**75.47**
Any food bought directly from a farmer or at a market	7.1	0	5.5	4.65	3.77
Food produced without synthetic fertilisers and pesticides	47.6	72.2	66.7	67.44	58.49
Any food produced without the use of genetically modified organisms	23.8	22.2	5.5	13.95	26.42
Any food without preservatives and artificial additives	33.3	22.2	11.1	13.95	35.85
Labels for organic products*					
healthy food	14.3	22.2	11.1	11.63	20.75
BIO (biological)	**52.4**	**63.9**	**66.7**	**72.09**	**50.94**
straight from nature	4.8	2.8	5.6	2.33	5.66
country/farmhouse	2.4	0	0	0	1.89
EKO (ecological)	**54.8**	**61.1**	**88.9**	**67.44**	**60.38**
organic	**9.5**	**19.4**	**44.5**	**23.26**	**16.98**
natural	11.9	0	5.6	0	11.32
preservative-free	19.1	8.3	0	4.65	16.98
The organic farming symbol **					
	73.8	**83.3**	**72.2**	**76.74**	**79.25**
Do you believe that organic food can play a role in improving the health and well-being of a child with ASD?					
yes	66.67	55.56	77.78	79.07	45.28
no	16.67	25.0	16.67	16.28	22.64
It's hard to say, I have no opinion	14.28	13.88	5.56	4.65	32.08
Have you noticed a correlation between consuming organic food and improved well-being for you and your child/children?***					
yes	42.86	19.44	11.11	48.866	16.98
no	33.0	69.44	61.11	46.51	52.83
It's hard to say, I have no opinion	9.52	0	16.67	11.63	9.43

* The number of responses does not add up to 100% because respondents could choose more than one answer; ** Respondent could choose only one answer; *** The number of responses does not add up to 100% because not all respondents provided an answer.

Source: own elaboration.

The symbol established by the European Commission, known as the 'Euro-Leaf', which guarantees that a given product comes from a certified organic farm, was recognised by the overwhelming majority of respondents (78.1% of those surveyed). It is possible that its distinctive shape, which appears prominently on the product packaging, or its presence in media advertisements contributed to this recognition. In a study conducted in 2016 among consumers visiting the 'Zielony Targ' market in Poznań (Poland), the highest number of respondents recognised the 'Euro-Leaf' among organic labels [27].

In the survey conducted by Woś et al. [28], nearly 85% of mothers with young children living in the eastern regions of Poland were able to identify the organic food logo. However, the authors of the study emphasised that only the survey sheets from respondents who indicated the correct definition of organic food were included; therefore, the group of surveyed women had greater knowledge about organic food than the average woman in Poland, which may have been related to a higher-than-average interest in this type of food. Similarly, in a group of 89 mothers with preschool-age children, a significant portion of the participants had correct knowledge about organic food (97%) and its labelling (76%) [29]. Undoubtedly, the arrival of a child in the family increases concern about nutrition, and parents may navigate the food market more consciously. However, in the IMAS study (2017), which included 518 Polish respondents, the familiarity with the logo of the organic food production certificate was very low (33%) [22].

The study confirmed that better knowledge of proper nutrition issues influences consumer awareness regarding organic food. Similarly, making changes in the diet of family members (e.g., special diets, avoiding allergens) can encourage parents to gain a deeper understanding of the food market. The vast majority of respondents belonging to the aforementioned groups believed that organic food could have a beneficial impact on people's well-being and improve the functioning of children with autism spectrum disorders (ASD) (Table 3).

Responses to the question, 'Have you noticed a correlation between consuming organic food and improved well-being for you and your child/children?' varied. Parents whose children were on an elimination diet more often than other respondents perceived a connection between improved well-being and the inclusion of organic products in their diet. Many people felt that it was difficult to assess whether the improvement in their child's functioning was solely due to proper nutrition, as children with ASD undergo multifaceted therapy (psychological, educational, pharmacological, etc.). Some respondents indicated that such an assessment would be possible after a longer period of regular consumption of organic food; however, they did not have sufficient financial resources for this.

The economic situation and the price of products are among the most important factors influencing consumers' purchasing decisions. When asked how much higher a price the respondents were willing to pay for an organic product compared to the same non-organic one, the largest number of respondents answered that it was a maximum of 10–20% (Table 4). The structure of responses varied and did not depend on the household income of the respondents or their level of satisfaction with their financial situation. The individual views of parents regarding organic food likely influenced this. Confirmation of this can be seen in the distribution of statements such as 'I will buy an organic product regardless of the price', where the highest percentage of respondents providing such an answer were individuals with lower or medium incomes. This is justified by parents' concerns for their children's health and the desire to instil good eating habits in them. As can be seen, this is a strong motivation that partially offsets the significance of a family's weaker financial status.

In the study by Kułyk and Michałowska [30], involving a group of 302 randomly selected respondents from the Lubuskie province of Poland, 38% of those surveyed expressed no willingness to pay a higher price for organic food. In contrast, those who declared a willingness to pay a higher price (102) were ready to incur an expense higher by 5–10%. Only a small percentage of individuals (4%) were willing to accept a higher price in the range of an extra 41–45% [30]. Although fruits and vegetables are the best-selling organic food products in Poland, the overall acceptance of price increases for organic apples in the study by Kazimierczak et al. [31] was about 20% (89% of responses). Similarly, in the study by Hermaniuk, the price difference accepted by the largest number of respondents between organic and conventional food was 20% [32]. The willingness to pay a higher price for organic food varies between countries, for instance, consumers from Germany and the United Kingdom are willing to pay up to 50% more, while Italians are even willing to pay up to 100% more [30].

The price of food also did not have a significant impact on the purchasing decisions of the surveyed parents regarding the choice between a foreign product with an organic certificate and a domestic non-organic one (Table 4). The respondents' decisions were largely dependent on the type of product and its origin: a large percentage of respondents would choose food produced in Poland.

The authors indicated [26, 33] that for Polish consumers, the idea of organic food production is closely related to the concept of traditional food production and is associated with the desire to shorten the production and distribution chain. The place of sale, product origin, and the structure of the assortment are important. Similarly, in a study conducted in Spain, consumers valued regionally produced tomatoes the most, especially ribbed organic tomatoes, provided they were produced on a national scale, taking into account a short supply chain [34]. Other studies have shown that for some consumers, geographical proximity (localness) is more important than the organic production system [35, 36].

An attempt was made to investigate the significance of various factors influencing respondents' purchase of organic food (Table 5). A Likert scale was used for this purpose, where 5 indicated very important, 4 – important, 3 – minimally important, 2 – neither important nor not important, and 1 – not important. For all parents, regardless of their level of knowledge about proper nutrition or the use of elimination diets for their children, the most important factors considered when purchasing organic food were health concerns and the safety of such food, which respondents associated with a low content of chemicals. The respondents were interested in products with simple ingredients, low levels of processing, and the nutritional content of the food they purchased. The positive impact of organic food on health as a key motivation for purchasing organic products is confirmed in the literature. Numerous studies conducted among various groups of respondents indicate that health benefits are the most important rationale for purchasing organic food [27–29, 32, 33, 37–39].

Table 4. Structure of responses depending on the respondents' income [%]

Question	Answer	Average monthly net income of a household per person							Level of satisfaction with income				
		1000 PLN or less *n* = 3	1001–2500 PLN *n* = 20	2501–3500 PLN *n* = 10	3501–5000 PLN *n* = 13	>5000 PL*N* *n* = 15	no data *n* = 35	total *n* = 96	very satisfied *n* = 8	satisfied *n* = 43	neither satisfied nor dissatisfied *n* = 33	dissatisfied *n* = 12	total *n* = 96
If you had the choice between a domestic product without an organic production certificate and the same foreign organic product, which one would you buy?	domestic	66.7	40.0	50.0	15.4	53.3	34.3	38.5	50	37.2	33.3	50	38.5
	foreign organic	0	0	0	7.7	0	11.4	5.2	12.5	2.3	6.1	8.3	5.2
	depends on the type of product	33.3	45.0	40.0	46.1	40.0	40.0	41.7	37.5	44.2	45.4	33.3	41.7
	depends on the price difference between the two products	0	10.0	10.0	23.1	6.7	11.4	11.5	0	11.6	12.1	8.3	11.5
	cheaper	0	5.0	0	7.7	0	2.8	3.0	0	4.6	3.0	0	3.0
How much higher a net price are you willing to pay for an organic product compared to the same product without an organic production certificate?	10% more	33.3	30.0	20.0	7.7	40.0	25.7	25.0	12.5	30.2	18.2	33.3	25
	20% more	33.3	35.0	10.0	46.1	26.7	20.0	27.1	25	27.9	24.2	33.3	27.1
	30% more	0	5.0	30.0	15.4	6.7	11.4	11.5	12.5	14.0	12.1	0	11.5
	50% more	0	5.0	0	7.7	6.7	5.7	6.3	0	7.0	6.1	8.3	6.3
	100% more	0	0	0	0	6.7	0	1.0	12.5	0	0	0	1.0
	I will buy the organic product regardless of the price	0	15.0	20.0	7.7	13.3	20.0	15.6	25	14.0	18.2	8.3	15.6
	I will always buy the cheaper one	33.3	10.0	20.0	15.4	0	17.1	13.5	12.5	7.0	21.2	16.7	13.5

Source: own elaboration.

Table 5. Impact of the importance of factors on the purchase of organic food by respondents based on their level of knowledge about proper nutrition and the application of a special diet for children with ASD.

DISTINGUISHING FEATURE		Total (child on **a diet**) *n* = 43			*good* *n* = 8			*adequate* *n* = 18			*insufficient* *n* = 17		
	R	***M***	**Md**	***SD***	***M***	**Md**	***SD***	***M***	**Md**	***SD***	***M***	**Md**	***SD***
care for health	2	4.65	5	0.78	4.13	5	1.46	4.72	5	0.57	4.82	5	0.39
chemical content	1	4.88	5	0.33	4.86	5	0.38	4.89	5	0.32	4.88	5	0.34
safety	4	4.54	5	0.81	4.29	5	1.11	4.56	5	0.92	4.63	5	0.50
GMO-free	8	4.11	5	1.28	3.71	4	1.11	4.14	5	1.41	4.25	5	1.24
degree of processing	6	4.40	5	0.84	4.00	4	1.15	4.44	5	0.78	4.53	5	0.74
taste, smell, appearance	7	4.23	4	0.90	4.57	5	0.53	4.06	4	1.09	4.27	4	0.80
simple ingredients	3	4.59	5	0.77	4.43	5	0.98	4.50	5	0.86	4.75	5	0.58
nutrient content	5	4.49	5	0.72	4.43	5	0.79	4.41	5	0.87	4.60	5	0.51
environmental care	10	3.87	4	0.99	3.71	3	0.95	4.00	4	1.12	3.79	4	0.89
animal welfare	9	3.92	4	1.00	4.14	4	0.90	3.82	4	1.24	3.93	4	0.73
medical recommendations	11	3.82	4	1.30	3.29	4	1.25	3.83	4	1.34	4.07	4.5	1.27
fashion, trends	13	1.74	1	1.06	1.57	1	0.79	1.73	1	0.96	1.89	1	1.45
curiosity	12	2.12	2	1.22	2.50	2.5	1.52	2.07	2	1.10	2.00	1.5	1.28
DISTINGUISHING FEATURE		Total (child **not on a diet**) *n* = 53			*good* *n* = 10			*adequate* *n* = 18			*insufficient* *n* = 25		
	R	***M***	**Md**	***SD***	***M***	**Md**	***SD***	***M***	**Md**	***SD***	***M***	**Md**	***SD***
care for health	1	4.56	5	0.78	4.75	5	0.46	4.54	5	0.88	4.50	5	0.83
chemical content	2	4.55	5	0.80	4.88	5	0.35	4.36	5	0.93	4.55	5	0.83
safety	3	4.28	4	0.86	4.25	5	0.71	4.25	5	0.97	4.32	5	0.89
GMO-free	8	3.73	4	1.40	3.88	4	1.36	3.00	5	1.58	4.15	5	1.14
degree of processing	5	4.19	4	0.86	4.25	4	0.46	4.00	5	1.04	4.30	5	0.86
taste, smell, appearance	7	3.95	4	1.05	4.00	5	0.76	3.62	4	1.39	4.17	4	0.86
simple ingredients	4	4.27	5	0.96	4.63	5	0.52	4.39	5	0.74	4.05	5	1.19
nutrient content	6	4.14	4	0.98	4.25	5	0.71	3.79	5	0.97	4.35	5	1.04
environmental care	9	3.66	4	0.81	4.13	3	0.35	3.42	4	0.91	3.63	4	0.83
animal welfare	10	3.45	3.5	0.76	3.50	4	0.76	3.25	4	0.75	3.56	4	0.78
medical recommendations	11	3.40	4	1.15	2.75	4	1.16	3.15	4	1.21	3.84	4.5	0.96
fashion, trends	13	2.14	2	0.99	1.50	1	0.93	2.31	1	1.03	2.33	1	0.90
curiosity	12	2.53	3	0.88	1.88	2.5	0.83	2.62	2	0.96	2.80	1.5	0.68

M – average (mean); Md – median; SD – standard deviation; R – ranking

Source: own elaboration.

Concerns about the natural environment and animal welfare were of lesser importance to parents as motivations for making consumption decisions regarding organic products (median 3 or 4) (Table 5). A similar consumer profile emerges from research conducted by Hansen et al. [40]: egoistic motivations, such as concern for health, have a greater influence on the purchase of organic food than altruistic motivations focused on environmental values. According to Średnicka-Tober et al., social issues have a minimal impact on consumers' decisions regarding the purchase of organic food products [20].

Regarding the relationship between respondents' level of knowledge about proper diet and nutrition and the assessment of the importance of various factors influencing their purchasing decisions (such as concern for health, chemical content, safety, absence of GMOs, degree of processing, taste/smell/appearance, concern for the environment, concern for animal welfare, medical recommendations, fashion/trends, and curiosity), a weak but noticeable negative correlation was found between respondents' knowledge (both in terms of self-assessed knowledge: $r = -0.291$, and the score achieved by respondents in the knowledge test: $r = -0.278$) and the importance placed on medical recommendations. Conversely, a high level of correlation was found between the importance attributed to nutritional ingredients and the assessment of the significance of taste/smell/appearance ($r = 0.586$), concern for animal welfare and concern for the environment (r = 0.726), as well as curiosity and fashion ($r = 0.695$).

Parents participating in current study had the opportunity to indicate other factors that were important to them when purchasing organic food. Among the responses provided by the participants were comments regarding supporting producers of high-quality food, particularly local producers: 'I am happy if I can buy something of good quality locally'. Some parents raised issues of trust in the seller, the brand of the product, the organic certification, and also pointed to the necessity of using eco-friendly food packaging.

The organic food market has significant potential; however, there are many factors that prevent consumers from fully understanding the high value of organic food. A major influence on this phenomenon is the asymmetry of knowledge and information regarding production, certification, and the identification of such food. Table 6 presents the opinions of respondents on the aforementioned topics and the related reasons for not purchasing organic food.

Table 6. Barriers to purchasing organic food

If you do not purchase organic food or purchase it rarely, please indicate the reasons.	Number of responses %*
The price is too high	61
I don't believe that farmers and producers actually follow the principles of organic production completely	41
I don't trust the organisations that certify producers	23
I don't see a difference in the quality and taste of such food compared to conventional food	18
I have limited access to organic food	17
I don't understand why organic food is more expensive than conventional food	11
I cannot distinguish organic food from conventional food	6
I don't know where I can buy it	4
I am not interested in organic food	3

* The number of responses does not add up to 100% because the respondent could select more than one answer.

Source: own elaboration.

The main reason why respondents do not buy organic food or buy it infrequently is definitely the price. The price difference between organic and conventional products, in some cases reaching up to 200%, is one of the main obstacles to the popularisation of such products in Poland and worldwide [30–32,37,41]. In a study by Bryła involving 1,000 Polish consumers, both low-income and high-income respondents cited high prices as a barrier to purchasing organic food [38].

A large percentage of surveyed parents indicated a lack of trust in those responsible for the various stages of organic product production ('From farm to table'), including the certification process (Table 6). Issues with trust and a sceptical attitude towards control systems in organic farming are more noticeable in developing countries than in those with higher economic status. In a study involving respondents from Kosovo, certification was the factor most strongly influencing their attitudes towards organic food [39]. Acceptance of certification by consumers is crucial for building trust and consuming organic food products, as many attributes of food that consumers seek and are willing to pay a premium for are not visible. The identification and validation of such food products are possible only by reducing the information asymmetry between producers and consumers, which can be achieved by ensuring certification by the appropriate certifying body. For consumers in Kosovo [39], an independent international certifying body was considered more credible than a national one.

More than 30% of parents do not appreciate the beneficial properties of organic food, which is likely due to insufficient knowledge about it (Table 6).

Respondents were asked about their sources of information regarding organic food, their purchasing locations, and shopping preferences (Table 7). The main source of knowledge for the surveyed parents is the Internet, followed by advice from doctors or dietitians. Interpersonal relationships also play an important role in providing information. The study indicates that respondents take into account the opinions of family and friends. This may be because they are more interested in learning about products recommended by trusted individuals. Other authors also highlight the dominant role of the Internet as a source of knowledge about organic food [29,33,43].

Table 7. Selected dietary behaviours of respondents related to organic food consumption (% of responses)

Analysis category	Verified Options	% of responses *
Explored sources of information about organic food	Internet	88.5
	doctor, dietitian	29.2
	family, friends	24.0
	television, radio programs	15.6
	advertising campaigns	12.5
	press	12.5
	food fairs	11.5
	sellers	4.2
Place of purchase for organic food	organic food store	42.7
	online store	38.5
	supermarket	36.5
	market, bazaar	30.2
	organic farm	21.9
	I do not purchase organic food	22.9
Selected groups of organic food products	fruits and vegetables	68.8
	eggs	64.6
	grain products (bread, groats, pasta, cereal)	44.8
	seeds, grains, nuts	35.4
	meat, meat products	34.4
	milk, dairy products or dairy alternatives	33.3
	fats	31.3
	spices	24.0
	fruit and vegetable preserves	21.9
	spreads and flavoured butters	18.8
	beverages and juices	15.6
	fish and fish products	14.6
	baby food	12.5
	confectionery	6.3

* The number of responses does not add up to 100% because the respondent could select more than one answer.

Source: own elaboration.

The surveyed parents make purchases of organic food in various places, most often in stores specialising in the sale of such products, but also in brick-and-mortar supermarkets and online stores. One-fifth of respondents chose direct contact with organic producers, and nearly one-third make purchases at markets. However, there is a question about whether food bought in such places (markets, bazaars) is truly organic, or whether respondents, due to a lack of sufficient knowledge, mistakenly identify the purchased products as organic.

Among organic food products, respondents most often include vegetables and fruits, eggs, and grain products in their shopping baskets (Table 7). The frequency of purchasing certain products such as meat and meat products, milk and dairy products, seeds and nuts, as well as edible fats is at a similar level (around 30%). These choices are not surprising, as these groups of products form the basis of a human diet. Moreover, certain organic products may be more or less available. Parents least frequently buy confectionery products, which is likely due to health concerns, or they may prepare such products at home. Other authors present similar purchasing preferences for organic products [20, 27, 29–32].

CONCLUSIONS AND FUTURE PERSPECTIVES

The aim of the study was to analyse the perception of organic food by parents of children with autism spectrum disorders. A higher level of awareness regarding the beneficial properties of organic food and the ability to identify it is noticeable among respondents with greater knowledge about proper nutrition and those following elimination diets compared to other parents. The most common reason for interest in organic products remains health-related concerns, while the main barrier to consuming organic food is its high price and lack of trust in organic food producers and the certification system. Environmental motivations are considered less important to consumers than health-related impulses, and the willingness to pay a higher price for organic products mainly depends on the type of product and its origin.

In light of the collected data, it seems reasonable to undertake actions aimed at raising parents' knowledge regarding proper nutrition and organic food production. When a child receives a diagnosis of an autism spectrum disorder, parents should receive extensive support and care. This support should include, among other things, guidance on the child's therapeutic activities, opportunities to strengthen parents in a new and challenging situation (e.g., where to seek psychological and psychiatric help or join parental support groups), and raising awareness – particularly crucial for children with autism – about the role of proper nutrition and food quality.

Initiatives to enhance knowledge about proper nutrition, diets, and the benefits of organic food should target the entire society, encompassing all age groups, from preschool to university students, as well as individuals who have already completed their education. Therefore, the government's decision to introduce a new subject – health education – in Polish schools starting in September 2025 is very timely. Students will learn about mental and physical health protection, proper nutrition, and sexual education, among other topics.

Additionally, marketing efforts should focus on clearly highlighting the distinguishing features of organic products compared to conventional ones, which would mitigate the perception of their high price. Furthermore, building proper communication and a trustful atmosphere between consumers and producers is essential.

Acknowledgement
This work presents the results of Project no 052/ZZZ/2023/POT, financed from a subsidy granted to the Krakow University of Economics.

REFERENCES

[1] **Solmi M., Song M., Keon Y.D., Lee S.W., Fombonne E., Kim M.S., Park S., Lee M.H., Hwang J., Keller J. et al. 2022.** Incidence, prevalence, and global burden of autism spectrum disorder from 1990 to 2019 across 204 countries. Molecular Psychiatry 27: 4172–4180. https://doi.org/10.1038/s41380-022-01630-7

[2] **Bhandari R., Paliwal J.K., Kuhad A. 2020.** Neuropsychopathology of Autism Spectrum Disorder: Complex Interplay of Genetic, Epigenetic, and Environmental Factors. In: M. Essa, M. Qoronfleh (eds), Personalized Food Intervention and Therapy for Autism Spectrum Disorder Management. Advances in Neurobiology. Springer, Cham: 97–141. https://doi.org/10.1007/978-3-030-30402-7_4

[3] **Roman P., Ruiz-Gonzalez C., Rueda-Ruzafa L., Cardona D., Requena M., Alarcon R. 2024.** Exposure to Environmental Pesticides and the Risk of Autism Spectrum Disorders: A Population-Based Case-Control Study. Medicina 60(3): 479–491. https://doi.org/10.3390/medicina60030479

[4] **Adams J., Baral M., Geis E., Mitchell J., Ingram J., Hensley A., Zappia I., Newmark S., Gehn E., Rubin R.A. et al. 2009.** The severity of autism is associated with toxic metal body burden and red blood cell glutathione levels. Journal of Toxicology 532640. https://doi.org/10.1155/2009/532640

[5] **Baj J., Flieger W., Flieger M., Forma A., Sitarz E., Skórzyńska-Dziduszko K., Grochowski C., Maciejewski R., Karakuła-Juchnowicz H. 2021.** Autism spectrum disorder: Trace elements imbalances and the pathogenesis and severity of autistic symptoms. Neuroscience & Biobehavioral Reviews 129: 117–132. https://doi.org/10.1016/j.neubiorev.2021.07.029

[6] **EFSA (European Food Safety Authority). 2018.** Monitoring data on pesticide residues in food: results on organic versus conventionally produced food. EFSA supporting publication EN-1397.30.

[7] **Leader G., Tuohy E., Chen J.L., Mannion A., Gilroy S.P. 2020.** Feeding problems, gastrointestinal symptoms, challenging behavior and sensory issues in children and adolescents with autism spectrum disorder. Journal of Autism and Developmental Disorders 50: 1401. https://doi.org/10.1007/s10803-019-04357-7.

[8] **Min G., Ling L., Qian Z., Li Ch., Ying D., Liyan L., Jianqing F., Xiaofan C.i, Qian Ch., Jie Ch., Hua W., Tingyu L. 2018.** Vitamin and mineral status of children with autism spectrum disorder in Hainan Province of China: associations with symptoms. Nutritional Neuroscience 23(10): 803–810. https://doi.org/10.1080/1028415X.2018.1558762

[9] **Lange E., Tarnowska K., Krusiec J., Gruczyńska E., Kowalska D., Kozłowska M. 2018.** Wpływ sposobu żywienia dzieci z zaburzeniami ze spektrum autyzmu na wybrane zachowania żywieniowe. Problemy Higieny i Epidemiologii 99(1): 12.

[10] **Belokoskova S.G., Tsikunov S.G. 2023.** Role of oxidative stress in the pathogenesis of autism spectrum disorders. Reviews on Clinical Pharmacology and Drug Therapy 21, 3: 215–230. https://doi.org/10.17816/RCF567781

[11] **Kopczyńska K., Kazimierczak R., Średnicka-Tober D., Barański M, Wyszyński Z., Kucińska K., Perzanowska A., Szacki P., Rembiałkowska E., Hallmann E. 2020**. The profile of selected antioxidants in two courgette varieties from organic and conventional production. Antioxidants 9: 404. https://doi.org/10.3390/antiox9050404

[12] **Hallmann E., Sabała P. 2020**. Organic and conventional herbs quality reflected by their antioxidant compounds concentration. Applied Sciences 10: 3468. https://doi.org/10.3390/app10103468

[13] **Glibowski P. 2020.** Organic food and health. Roczniki Państwowego Zakładu Higieny 71(2): 131. http://dx.doi.org/10.32394/rpzh.2020.0110

[14] **Neumeyer A.M., Gates A., Ferrone C., Lee H., Misra M. 2013.** Bone density in peripubertal boys with autism spectrum disorders. Journal of Autism and Developmental Disorders 43(7): 1623–1629. https://doi.org/10.1007/s10803-012-1709-3

[15] **Doaei S., Bourbour F., Teymoori F., Jafari F., Kalantari N., Abbas Torki S., Ashoori N., Nemat Gorgani S., Gholamalizadeh M. 2021.** The effect of omega-3 fatty acids supplementation on social and behavioral disorders of children with autism: a randomized clinical trial. Pediatric Endocrinology Diabetes and Metabolism 27(1): 12–18. https://doi.org/10.5114/pedm.2020.101806.

[16] **Średnicka-Tober D., Barański M., Seal C.J. et. al. 2016.** Higher PUFA and n-3 PUFA, conjugated linoleic acid, α-tocopherol and iron, but lower iodine and selenium concentrations in organic milk: a systematic literature review and meta- and redundancy analyses. British Journal of Nutrition 115(6): 1043–1060. https://doi.org/10.1017/S0007114516000349

[17] **Średnicka-Tober D., Barański M., Seal Ch. et. al. 2016.** Composition differences between organic and conventional meat: a systematic review and meat-analysis. British Journal of Nutrition 115(6): 994–1011. https://doi.org/10.1017/S0007114515005073

[18] **Presmanes A., Zuckerman K.E., Fombanne E. 2015.** Obesity and Autism. Pediatrics 136(6): 1051–1061. https://doi.org/10.1542/peds.2015-1437

[19] **Gawęcki J. (ed.). 2020.** Kwestionariusz do badania poglądów i zwyczajów żywieniowych oraz procedura opracowania danych KomPAN: wersja polskojęzyczna, wydanie drugie. Część C – Kwestionariusz do badania poglądów i zwyczajów żywieniowych dla osób w wieku od 16 do 65 lat wersja 1.2 – kwestionariusz do samodzielnego wypełnienia przez respondenta. Komitet Nauki o Żywieniu Człowieka Polskiej Akademii Nauk, Olsztyn.

[20] **Średnicka-Tober D., Kazimierczak R., Hallmann E. 2016.** Charakterystyka europejskich konsumentów żywności ekologicznej – motywy, działania i implikacje. Problemy Zarządzania 14, 1(58), 2: 100–108. https://doi.org/10.7172/1644-9584.58.7

[21] **Knech K., Gurwin A. 2019.** Rynek żywności ekologicznej. Edukacja dla Bezpieczeństwa 12, 4(45): 83–93.

[22] **IMAS International. 2017.** Żywność ekologiczna w Polsce 2017,Wrocław.

[23] **IMAS International. 2021.** Żywność ekologiczna w Polsce 2021, Warszawa.

[24] **Sharma, S.R, Gonda X., Tarazi F.I. 2018.** Autism Spectrum Disorder: Classification, diagnosis and therapy. Pharmacology & Therapeutics 190: 91–104. https://doi.org/10.1016/j.pharmthera.2018.05.007

[25] **Tarnowska K., Gruczyńska–Sękowska E., Kowalska D., Majewska E., Kozłowska M., Winkler R. 2023.** The opioid excess theory in autism spectrum disorders – is it worth investigating further? Critical Reviews in Food Science and Nutrition 63(19): 3980–3993. https://doi.org/10.1080/10408398.2021.1996329

[26] **Żakowska-Biemans S. 2012.** Czynniki warunkujące popyt na żywność ekologiczną w kontekście przeobrażeń rynku żywności ekologicznej w Polsce i innych krajach Europy. MRiRW, Warszawa – Falenty.

[27] **Baer-Nawrocka A., Szalaty N. 2017.** Produkty ekologiczne w opinii konsumentów i producentów w studium przypadku. Zagadnienia Ekonomiki Rolnej 4(353): 138–153.

[28] **Woś K., Dobrowolski H., Gajewska D., Rembiałkowska E. 2022.** Organic Food Consumption and Perception among Polish Mothers of Children under 6 Years Old. International Journal of Environmental Research and Public Health 19: 15196. https://doi.org/10.3390/ijerph192215196

[29] **Samolińska W., Kiczorowska B., Marzec A., Kowalczuk-Vasilev E., Klebaniuk R., Kwiecień M. 2017.** Żywność ekologiczna w opinii matek dzieci w wieku przedszkolnym w Lublinie. Problemy Higieny i Epidemioliologii 98(3): 266–170.

[30] **Kułyk P., Michałowska M. 2018.** Zachowania konsumentów na rynku żywności ekologicznej w świetle wyników badań empirycznych w województwie lubuskim. Handel Wewnętrzny 2(373): 270–282.

[31] **Kazimierczak R., Obidzińska J., Szumigaj B., Dobrowolski H., Rembiałkowska E. 2024.** Sustainable Foods: Consumer Opinions and Behaviour towards Organic Fruits in Poland. Sustainability 16: 3740–3761. https://doi.org/10.3390/su16093740

[32] **Hermaniuk T. 2018.** Postawy i zachowania konsumentów na rynku ekologicznych produktów żywnościowych. Handel Wewnętrzny 2(373):189–199.

[33] **Olech E., Kuboń M. 2015.** Motywy wyboru produktów ekologicznych przez konsumentów segmentu demograficznego z terenu Małopolski. Stowarzyszenie ekonomistów rolnictwa i agrobiznesu. Roczniki Naukowe 17(1): 164–169.

[34] **Bernabéu R., Brugarolas M., Martínez-Carrasco L., Nieto-Villegas R., Rabadán A. 2023.** The Price of Organic Foods as a Limiting Factor of the European Green Deal: The Case of Tomatoes in Spain. Sustainability 15: 3238–3253. https:// doi.org/10.3390/su15043238

[35] **Nestorowicz R., Pilarczyk B., Jerzyk E., Rogala A., Disterheft A. 2016.** Etnocentryzm konsumencki w ujęciu lokalnym jako czynnik rozwoju rynku żywności ekologicznej w Polsce. Roczniki Naukowe Stowarzyszenia Ekonomistów Rolnictwa i Agrobiznesu 6: 144–151.

[36] **Denver S., Jensen J.D., Olsen S.B., Christensen T. 2019.** Consumer Preferences for 'Localness' and Organic Food Production. Journal of Food Products Marketing 25: 668–689. https://doi.org/10.1080/10454446.2019.1640159

[37] **Soroka A., Mazurek-Kusiak A.K., Trafiałek J. 2021**. Organic Food in the Diet of Residents of the Visegrad Group (V4) Countries – Reasons for and Barriers to Its Purchasing. Nutrients 13: 4351. https://doi.org/10.3390/nu1312435

[38] **Bryła P. 2016.** Organic food consumption in Poland: Motives and barriers. Appetite 105: 737–746. https://doi.org/10.1016/j.appet.2016.07.012

[39] **Miftari I., Haas R., Meixner O., Imami D., Gjokaj E. 2022.** Factors Influencing Consumer Attitudes towards Organic Food Products in a Transition Economy – Insights from Kosovo. Sustainability 14: 5873–5887. https:// doi.org/10.3390/su14105873

[40] **Hansen T., Sørensenb M.I., Eriksen M.L.R. 2018.** How the interplay between consumer motivations and values influences organic food identity and behavior. Food Policy 74: 39–52. https://doi.org/10.1016/j.foodpol.2017.11.003

[41] **Kociszewski K., Sobocińska M., Krupowicz J., Graczyk A., Mazurek-Łopacińska K. 2023.** Changes in the Polish market for agricultural organic products. Economics and Environment 1(84): 259–286. https://doi.org/10.34659/eis.2023.84.1.547

[42] **Nagy L.B., Lakner Z, Temesi A. 2022.** Is it really organic? Credibility factors of organic food. A systematic review and bibliometric analysis. PLoS ONE 17(4): e0266855. https://doi.org/10.1371/ journal.pone.0266855

[43] **Cichocka I., Krupa J. 2016.** Znajomość żywności ekologicznej wśród mieszkańców województwa podkarpackiego. Handel Wewnętrzny 6(365): 32–46.