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Garlic Stick with Modified Cassava Flour, Isolated Soy Protein, Medan Anchovy, and Guar Gum Containing Protein and Calcium as an Alternative Snack for Children with Autism

Stik Bawang Kombinasi Modified Cassava Flour*,* Isolated Soy Protein*, Teri Medan, dan* Guar Gum *dengan Protein dan Kalsium sebagai Kudapan Alternatif bagi Anak Penyandang Autism*

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ABSTRACT

Background: Sensitivity to gluten and casein in children with Autism Spectrum Disorder (ASD) is crucial to symptom severity, making their elimination necessary. However, the rising prevalence of ASD in Indonesia is not matched by the recommended dietary consumption. A garlic stick formulated with Modified Cassava Flour (MOCAF), Isolated Soy Protein (ISP), medan anchovy, and guar gum offers a gluten- and casein-free alternative while serving as a potential source of protein and calcium for ASD children aged 4-9 years.
Objectives: To analyze the acceptability and nutrient content (protein and calcium) of garlic sticks made with MOCAF, ISP, medan anchovy, and guar gum.
Methods: This research was a pure experimental study using a Completely Randomized Design (CRD) with four treatments. F0 (0% MOCAF, 0% ISP, 0% medan anchovy, 0% guar gum), F1 (51% MOCAF, 9% ISP, 2% medan anchovy, 1% guar gum), F2 (42% MOCAF, 16% ISP, 4% medan anchovy, 2% guar gum), and F3 (32% MOCAF, 23% ISP, 6% medan anchovy, 3% guar gum). Organoleptic values were analyzed using Kruskal-Wallis and Mann-Whitney U test. Protein and calcium levels were assessed using the Kjeldahl method and Atomic Absorption Spectroscopy.
Results: F3 had the highest organoleptic acceptability. The protein and calcium content per 100 g of garlic stick were 8.26 g and 89.24 mg, respectively. A significant difference was observed between F0 and F3 flavor (p-value=0.004).
Conclusions: F3 was the optimal formula. One serving (55 g) met 11-18% of the protein requirement, while two servings were necessary to fulfill the calcium requirement for a single snacking occasion.

INTRODUCTION

Hypersensitivity or hyposensitivity to noise, texture, or smell, which influences food selectivity, is one of the characteristics of individual with Autism Spectrum Disorder (ASD)[1]. Sensitivity in this context refers to gluten and casein intolerance. Individuals with ASD cannot properly digest peptides (gluten and casein), which is believed to result in a buildup of peptides that may increase antibody levels against certain substances, including *anti-gliadin*, *anti-casein*, and *dipeptidyl peptidase 4-enzymes* that are essential for breaking down gliadin into peptides, such as *gliadinomorphin-7*, which has "opioid activity". This condition can lead to symptoms such as hyperactivity, repetitive behaviors, and concentration deficits during the learning process[2]. Additionally, gluten can induce systemic inflammation, including neuroinflammation in children with ASD[3]. According to a 2019 study by the World Health Organization (WHO), ASD affects at least 1 in 160 children globally[4]. Meanwhile, the Centers for Disease Control and Prevention (CDC) reported that the prevalence of ASD has been increasing, with an estimated incidence of 1 in 59 children in 2018[5]. However, the rising number of children with ASD contrasts with the low adherence to dietary recommendations[6].

Camelia, Wijayanti and Nissa (2019) revealed that parents of children with ASD at SLB ABC Bina Putera Ambarawa and SLBN Ungaran in Semarang Regency did not provide their children with the recommended diet due to concerns that their children might become sick or fussy, reluctance to refuse food from family members, unavailability of proper meals according to the dietary recommendations in their neighbourhood, and insufficient time for meal preparation[7]. Furthermore, the limited supply and lack of labelling significantly impact the consumption of gluten- and casein-free products[8]. A

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market survey by Nastiti and Christyaningsih (2019) indicated that this issue persists in Indonesia, where over 50% of market products contain wheat flour (which includes glutens), such as instant noodles, biscuits, snacks, bread, and garlic sticks[9]. This phenomenon contributes to the low adherence to gluten- and casein-free diets for children with ASD, as supported by Izzah, Fatmaningrum, and Irawan (2020) involving six ASD therapy centers in Surabaya, including SLB Autis Mutiara Hati Surabaya. Their study, which involved 100 children with ASD, found that only 37 children, 17 of whom were students at SLB Autis Mutiara Hati Surabaya were following a gluten- and casein-free diet[10]. In reality, adherence to dietary recommendations for children with ASD is closely linked to their symptoms[11].

Beyond gluten and casein, children with ASD are also prone to micronutrient deficiencies, including calcium deficiency, which is one of the adverse effects of a casein-free diet. Casein-rich foods, such as butter and milk are high in calcium[12]. Calcium plays a crucial role in nerve impulse transmission, and lower calcium levels increase the risk of symptoms such as anxiety, hypersensitivity, nervousness, irritability, and mental disorders[13]. Additionally, calcium helps prevent neurobehavioral changes, elevated blood lead levels, arsenic accumulation and toxicity related to ASD pathogenesis, and mitochondrial dysfunction[14]. A study conducted in Jordan on 22 male and 10 female children with ASD found significant cases of calcium deficiency[15].

These factors highlight the need for functional foods for children with ASD. Functional foods are those that provide essential nutrients while offering additional health benefits[16]. They can serve as preventive nutrition and can be derived from both animal and plant souces[17]. Gluten- and casein-free snacks that contain protein and calcium can be considered functional foods for children with ASD. One such snack is garlic sticks, a traditional flat stick-shaped pastry that is fried[18]. With their crunchy texture, salty flavor, and long shelf-life, garlic sticks are widely consumed, including by children[19]. Typically, garlic sticks are made with wheat flour as the primary ingredient. However, since children with ASD cannot consume gluten, an alternative is to use local ingredients, such as flour derived from gluten-free tubers like modified cassava flour (MOCAF) and from legumes such as isolated soy protein (ISP) in order to compensate for the protein lost from wheat flour elimination. Additionally, incorporating a casein-free animal protein source, such as medan anchovy, can enhance the micronutrient content, particularly calcium.

MOCAF is a type of flour made from fermented cassava tubers[20]. The fermentation process enhances viscosity, gelation ability, rehydration power, and solubility[21]. MOCAF shares many characteristics with wheat flour, such as its white color and fine texture, making it a suitable alternative[22]. ISP, a soybean derived flour, primarily consists of *glycinin* and *β-conglycinin*. It contains 90% protein and is commonly used as a dough binder that improves dough quality[22].

Medan anchovy is a small pelagic fish that inhabits sea waters and spoils quickly due to its small size[23]. It is short-bodied and white-colored[24]. Medan anchovy is rich in protein and calcium, making it a superior alternative to casein- containing dairy products. Medan anchovy is also affordable and widely available in Indonesia[25]. Meanwhile, guar gum, a white, water-soluble, nonionic polymer galactomannan extracted from guar beans (*Cyamopsistetra Gonoloba*)[26] acts as a binding agent that enhances dough texture and flexibility[27]. As a hydrocolloid, guar gum can replace gluten's role in gluten-free processed products[28].

Garlic sticks made from MOCAF, ISP, medan anchovy, and guar gum can be an appropriate snack for children with ASD aged 4-9 years. This age range is based on a study conducted by Kurnia and Muniroh (2018), which involved 16 children with ASD aged 4-6 years and 15 children with ASD aged 7-9 years in Surabaya. The study indicated a tendency toward picky eating behavior in this age group[29]. Another study by Bicer and Alsaffar (in Kurnia and Muniroh, 2018) found that children aged 4-18 years were typically categorized as picky eaters[29]. Thus, garlic sticks made from MOCAF, ISP, medan anchovy flour, and guar gum could serve as a gluten- and casein-free snack while fulfilling the protein and calcium needs of children with ASD. Accordingly, this study aims to analyze the impact of MOCAF, ISP, medan anchovy, and guar gum on the degree of likeability (texture, color, aroma, and flavor), as well as to evaluate the protein and calcium levels in garlic sticks made from these ingredients.

METHODS

This study employed a true experimental design research with a completely randomized design (CRD). It involved four treatment groups with varying ingredient compositions as followed: F0 (control) with 0% MOCAF substitution, 0% ISP substitution, 0% medan anchovy flour addition, 0% guar gum addition; F1 with 51% MOCAF substitution, 9% ISP substitution, 2% medan anchovy flour addition, 1% guar gum addition; F2 with 42% MOCAF substitution, 16% ISP substitution, 4% medan anchovy flour addition, 2% guar gum addition; and F3 with 32% MOCAF substitution, 23% ISP substitution, 6% medan anchovy flour addition, 3% guar gum addition. This research was conducted from December 2023 to June 2024, with the primary experimental procedures carried out at the Food Processing Laboratory, Faculty of Public Health, Airlangga University. The organoleptic analysis involved a hedonic test, which was statistically analyzed using the Kruskal-Wallis and Mann-Whitney U tests on 35 children with ASD and their parents or legal guardians at SLB Autis Mutiara Hati Surabaya and Sidoarjo, who participated as untrained panelists. The protein and calcium contents of the garlic sticks were analyzed in the Nutrients Analysis Laboratory, Faculty of Public Health, Airlangga University. The protein content was measured using the *Kjeldahl* method, while the calcium content was analyzed using the *Atomic Absorption Spectroscopy* (AAS) method. This research received ethical approval from the Health Research Ethical Clearance Commission of the Faculty of Public Health, Airlangga University, under reference number 110/EA/KEPK/2024, issued on April 23, 2024.

Cooking Utensils and Ingredients for Formulation

The ingredients used in this study included all-

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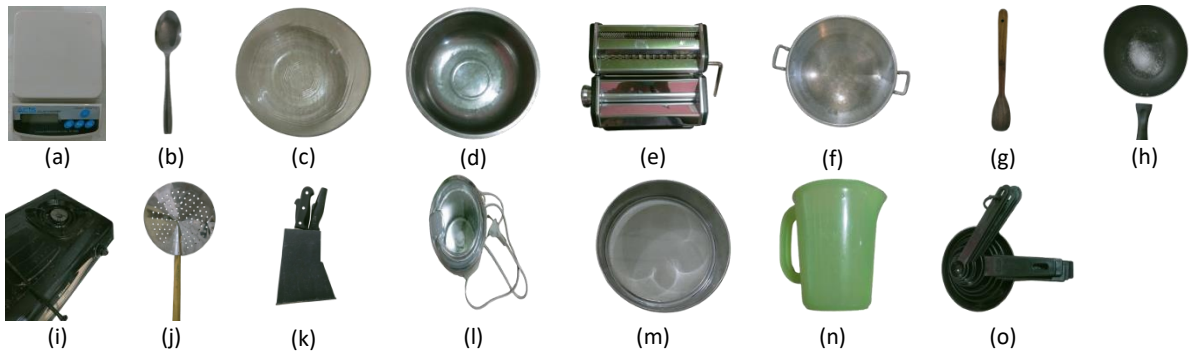
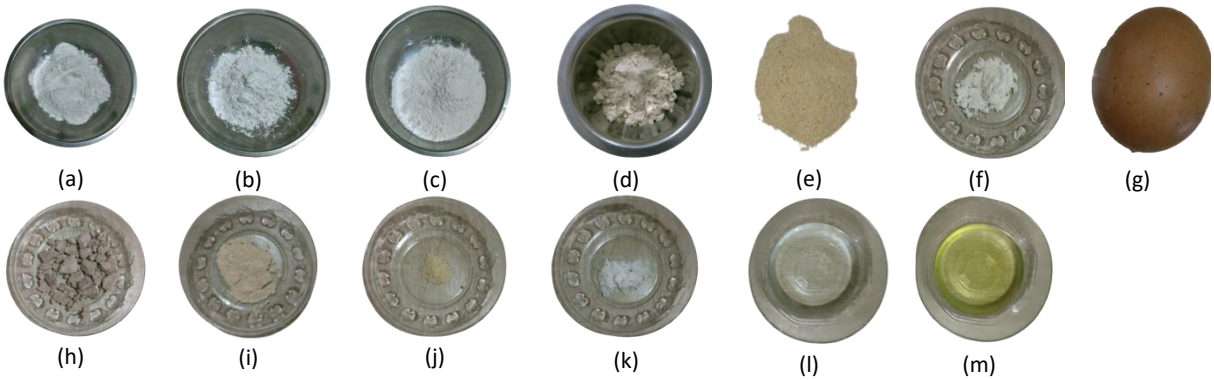
purpose flour, tapioca flour, MOCAF, ISP, medan anchovy flour, guar gum, free-range egg, shallot powder, garlic powder, beef stock powder, salt, water, and palm oil (Figure 1). The cooking tools used included an electronic kitchen scale, spoon, glass bowl, stainless kitchen bowl, molding machine (manual noodle maker), wok, spatula, frying pan, stove, oil strainer, knife set, flour mill, 80-mesh flour sieve, measuring cup, and measuring spoon.



a) Electronic Kitchen Scale (b) Spoon (c) Bowl (d) Stainless Kitchen Bowl (e) Molding Machine (f) Wok (g) Spatula (h) Frying Pan (i) Stove (j) Oil *Strainer* (k) Knife Set (l) *Flour Mill* (m) 80-*Mesh* Flour Sieve (n) Measuring Cup (o) Measuring Spoon

Figure 1. Cooking Utensils



a) All-purpose Flour (b) Tapioca Flour (c) MOCAF (d) ISP (e) Medan Anchovy Flour (f) Guar Gum (g) Free-range Egg (h) Shallot Powder (i) Garlic Powder (j) Beef Stock Powder (k) Salt (l) Water (m) Palm Oil

Figure 2. Ingredients

The garlic stick formula in this study was a modification of the garlic stick recipe by Sa'diyah and Tazkiyah (2023)[30]. The modifications involved replacing all-purpose flour and tapioca flour with MOCAF and ISP, as well as adding medan anchovy flour and guar gum. Table 1 presents the research formula.

Table 1. Raw Dough Formula of Garlic Stick

Ingredients	Treatment			
	F0	F1	F2	F3
All-purpose Flour (g)	250	0	0	0
Tapioca Flour (g)	65	0	0	0
MOCAF (g)	0	265	220	175
ISP (g)	0	45	85	125
Medan Anchovy Flour (g)	0	10	20	30
Guar Gum (g)	0	5	10	15
Free-range egg (g)	50	50	50	50
Shallot Powder (g)	5	5	5	5
Garlic Powder (g)	5	5	5	5
Beef Stock Powder (g)	5	5	5	5
Salt (g)	5	5	5	5
Water (ml)	125	125	125	125
Palm Oil (ml)	0	0	0	0

MOCAF (Modified Cassava Flour), ISP (Isolated Soy Protein)

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Preparation of Medan Anchovy Flour

The preparation of medan anchovy flour began by soaking the anchovy in boiling water for 10 minutes to reduce its salty flavor. The soaked anchovy was then roasted over low-heat for 30 minutes until completely dried. Once roasted, it was blended using a flour mill and sifted using an 80-mesh flour sieve.

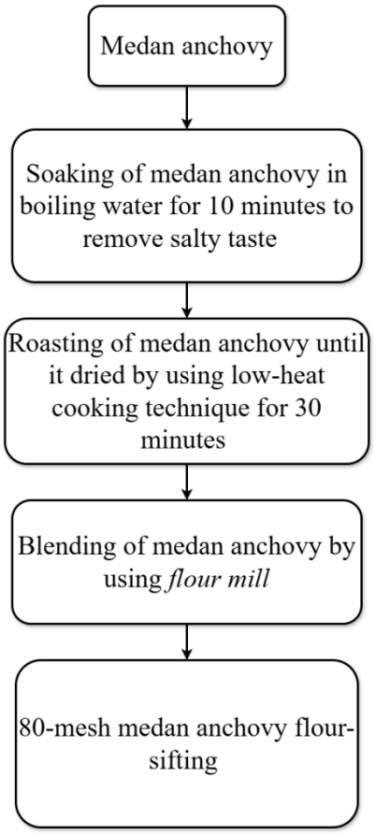

Figure 3. Flow Diagram of Medan Anchovy Flour Preparation

(a) (b) (c) (d)

(a) Soaking of Medan Anchovy (b) Roasting of Medan Anchovy (c) Blending of Medan Anchovy
(d) Medan Anchovy Flour-Sifting Result

Figure 4. Preparation of Medan Anchovy Flour

Preparation of Garlic Stick

The garlic stick preparation followed a modified version of Sa'diyah and Tazkiyah (2023) recipe[30]. All dry ingredients were mixed before adding the wet ingredients. The preparation process was conducted gradually, using separate cooking tools for F0 and the modified formulas (F1-F3) preparation to prevent cross-contamination with gluten-containing ingredients.

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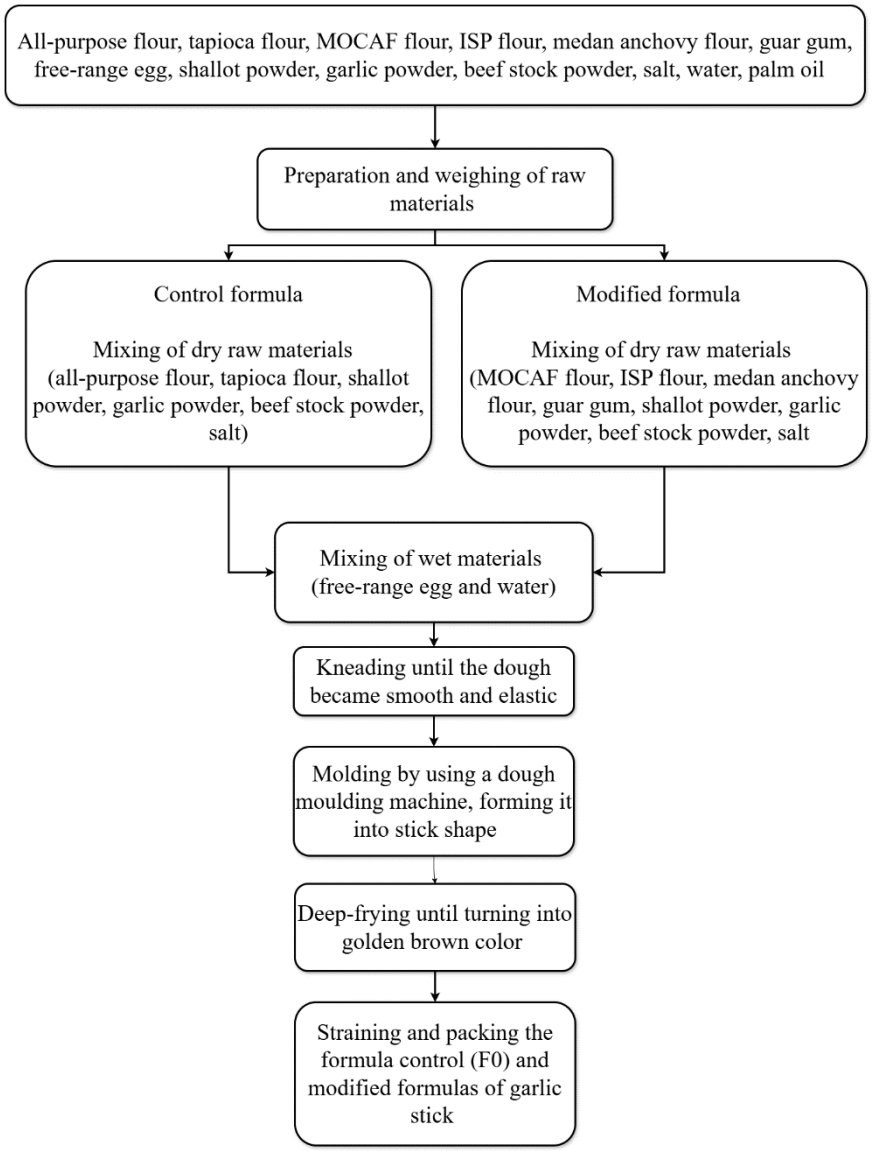
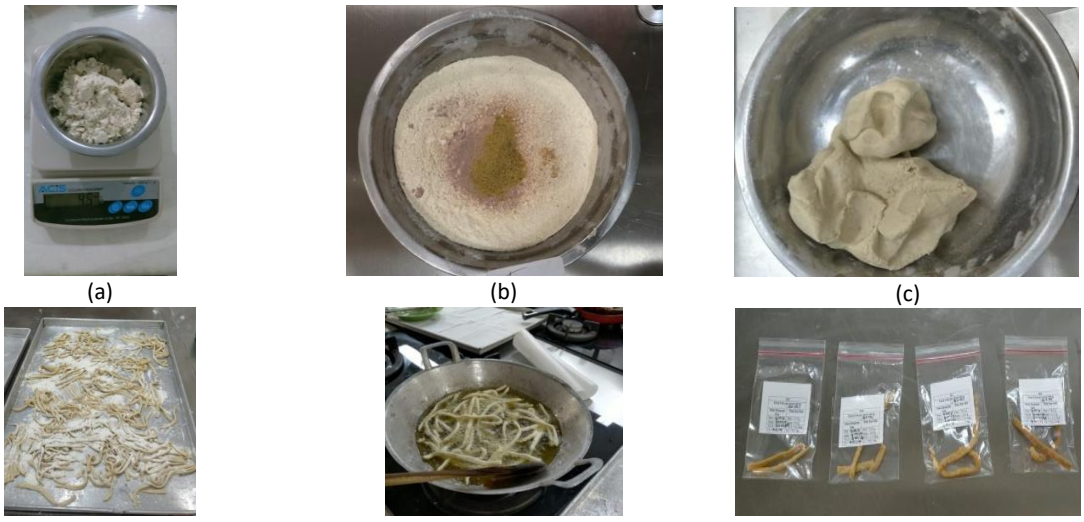


Figure 5. Flow Diagram of Garlic Stick Preparation



(a) Weighing of Raw Materials (b) Mixing of All Ingredients (c) Kneading of the Dough (d) Molding of the Dough (e) Deep-frying of Garlic Stick (f) Packing of Garlic Stick for Hedonic Test

Figure 6. Garlic Stick Preparation

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Organoleptic Analysis

The organoleptic analysis of garlic stick samples was conducted using a hedonic test involving 35 ASD children aged 4-9 years and their parents/legal guardians at SLB Autis Mutiara Hati Surabaya and Sidoarjo as untrained panelist. The inclusion criteria were 1) male and female ASD children aged 4-9 years at SLB Autis Mutiara Hati Surabaya and Sidoarjo; 2) ability to communicate and interact socially; 3) normal sense of flavor; 4) prior reading of the Explanation Before Research (PSP) section; 5) completion of the consent form for participation; 6) willingness to participate as a panelist; and 7) accompaniment by teachers/parents/legal guardians during the hedonic test. The exclusion criteria included ASD children and parents/legal guardians with a history of allergies or a strong distaste for specific ingredients, particularly egg and medan anchovy, as determined through interviews with parents/legal guardians.

The panelists assessed four parameters: color, texture, aroma, and flavor using a 5-point hedonic scale (1=very dislike, 2=dislike, 3=slightly like, 4=like, and 5=very like). ASD children assessed F1, F2, and F3, while parents/legal guardians evaluated F0, F1, F2, and F3. Each sample weighed 3 g. The organoleptic analysis of F0 was not conducted on ASD children due to its gluten content from all-purpose flour.

Data Analysis (α=0.05)

The hedonic test results were statistically analyzed using the Kruskal-Wallis Test (α=0.05) in IBM SPSS Statistics 20 to determine differences among garlic stick formulas. If significant differences were found, a further analysis using the Mann-Whitney U Test (α=0.05) was conducted to identify specific formula groups with significant variations.

Nutrient Content Analysis

The theoretical nutrient content measurement was conducted through a literature study based on TKPI (2019)[31]. The laboratory analysis of protein content used the *Kjeldahl* method, while the calcium content was measured using the *Atomic Absorption Spectroscopy* (AAS) method. The protein and calcium content analyses were representative of 100 g of garlic stick samples.

RESULTS AND DISCUSSIONS

Organoleptic Analysis

The organoleptic analysis conducted through a hedonic test assessing color, texture, aroma, and flavor indicated that F3 (175 g MOCAF, 125 g ISP, 30 g medan anchovy flour, 15 g guar gum) was the most preferred formula among both parents/legal guardians and ASD children panelists. The nutrient contents of F0 (control formula) and F3 (optimum formula) were analyzed using the *Kjeldahl* method for protein and *Atomic Absorption Spectroscopy* (AAS) for calcium. This aimed to evaluate the differences and impact of the modified ingredients on nutrient composition.

(a) (b) (c) (d)

(a) F0 Final Product (b) F1 Final Product (c) F2 Final Product (d) F3 Final Product

Figure 7. Final Products of Garlic Stick

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Color

Color is the first visible organoleptic parameter which uses eyes as the sensory organ in the visual system to do the analysis. The more interesting the color, the higher the consumer interest in tasting the products served[32]. Color can be affected by natural pigments such as carotenoids, synthetic colorants, and chemical reactions[33]. As shown in Table 2, F3 was the most preferred formula among parents/legal guardians (mean rank=3.62). However, Table 3 indicates that ASD children panelists favored F2 (mean rank=3.80), suggesting differing preferences between the groups[34].

Despite this variation, Kruskal-Wallis test results showed no significant differences in color preferences between F2 and F3, as the ISP and medan anchovy flour proportions differed by only 2%. The color of the garlic stick resulted from the Maillard reaction, a process that occurs when carbohydrates react with primary amino groups during frying, leading to protein denaturation and brownish hue[33]. Higher ISP and medan anchovy flour proportions, which increase protein content, contributed to the darker color of the garlic stick.

Table 2. Results of Garlic Stick Hedonic Test of Parents/Legal Guardians Panelists at SLB Autis Mutiara Hati Surabaya and Sidoarjo, East Java, Indonesia

Parameter	Mean Rank			
	F0	F1	F2	F3
Color	3.28	3.17	3.25	3.62
Texture	3.45	3.62	3.28	3.68
Aroma	3.82	3.62	3.94[b]	4.11[b]
Flavor	3.94	4.00	4.25	4.45[ab]

SLB (School for Students with Special Needs), [a]) compared with F0, [b]) compared with F1

Table 3. Results of Garlic Stick Hedonic Test of ASD Children Panelists at SLB Autis Mutiara Hati Surabaya and Sidoarjo, East Java, Indonesia

Parameter	Mean Rank		
	F1	F2	F3
Color	3.57	3.80	3.74
Texture	3.11	3.65[a]	3.80[a]
Aroma	3.31	3.65	3.74
Flavor	3.74	4.22[a]	4.17[a]

ASD (Autism Spectrum Disorder), [a]) compared with F1

Texture

Texture is an organoleptic characteristic perceived through touch and is closely linked to crispness and brittleness[35]. Food texture is influenced by its water, fat, carbohydrate, and protein content, as well as the amylopectin component of its starch[28]. Table 2 and Table 3 indicate that both parents/legal guardians (mean rank=3.68) and ASD children panelists (mean rank=3.80) found F3 to have the most favorable texture. Statistical analysis using Kruskal-Wallis and Mann-Whitney U tests revealed significant differences in texture preferences, particularly between F1 and F2 (*p-value*=0.024) and between F1 and F3 (*p-value*=0.008).

The texture of the garlic stick was influenced by the proportions of MOCAF, ISP, and guar gum. MOCAF contains 78.80%-79.06% amylopectin, which enhances water-binding capacity, thereby increasing crispness[36]. ISP contributes to texture stabilization by binding water[37], while guar gum facilitates water retention by preventing excessive moisture loss due to starch gelatinization that affects the absorption of intermolecular fractions into hydrogen bonds[38].

Aroma

Aroma, perceived through the olfactory system, plays a crucial role in food acceptability. A product's aroma can influence consumer perception and appetite[33]. As shown in Table 2 and Table 3, F3 had the highest aroma preference among both parents/legal guardians (mean rank=4.11) and ASD children panelists (mean rank=3.74). Statistical analysis indicated significant differences in aroma preferences between F1 and F2 (*p-value*=0.049) and between F1 and F3 (*p-value*=0.004), particularly in the hedonic test involving parents/legal guardians.

The distinctive aroma of F3 was primarily influenced by its medan anchovy flour content (30 g), which contributed to its characteristic scent. Aroma development occurs through protein breakdown, leading to the release of glutamic acid. Previous research on fish stick formulations has shown that higher fish flour content enhances aroma, increasing consumer acceptance[33].

Flavor

Flavor, perceived through taste, is a key determinant of product acceptability. It results from the interaction of various ingredients, creating a distinct sensory experience[35] that influence consumers' acceptability. Products with good nutrient content but poor flavor are difficult to gain popularity and to stay in the market[33]. Table 2 indicates that parents/legal guardians rated F3 highest in flavor (mean rank=4.45), while Table 3 shows that ASD children panelists preferred F2 (mean rank=4.22).

Kruskal-Wallis and Mann-Whitney U test results identified significant differences in flavor preferences, with notable contrasts between F0 and F3 (*p-*

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value=0.004) and between F1 and F3 (*p-value*=0.011) according to parents/legal guardians, as well as between F1 and F2 (*p-value*=0.025) and between F1 and F3 (*p-value*=0.034) according to ASD children panelists. According to Lianitya *et.al.* (in Ramadhan, Nuryanto and Wijayanti, 2019), differences in preference may stern from the unique taste of medan anchovy, which is relatively uncommon in commercially available products[25]. However, another scientific study conducted by Fera, Asnani and Asyik (2019) indicated that increasing fish flour content in fish stick formulations enhances flavor acceptance[33].

Nutrient Content Analysis

F3 contained the highest protein content (14.99 g/portion), primarily influenced by its medan anchovy flour and ISP content. Medan anchovy flour contributes 93 g of protein per 100 g, while ISP provides 88.3 g of protein per 100 g[31]. Additionally, theoretical calculations indicated that F3 contained 129.66 mg of calcium per portion, with medan anchovy flour being the primary source (2849 mg of calcium per 100 g)[31].

Table 4. Comparison of Protein and Calcium Levels between Control Formula (F0) and Optimum Formula (F3) of Garlic Stick according to Theoretical (TKPI) and Laboratory (*Kjeldahl* and AAS) Analysis

Formula	Theoretical				Laboratory			
	Protein (100 g)	Protein (55 g)	Calcium (100 mg)	Calcium (55 mg)	Protein (100 g)	Protein (55 g)	Calcium (100 mg)	Calcium (55 g)
F0	7.96	4.38	42.56	23.40	3.17	1.74	0.09	0.05
F3	27.27	14.99	235.75	129.66	8.26	4.54	89.24	49.08

TKPI (Indonesian Food Composition Table), AAS (*Atomic Absorption Spectroscopy*)

Nutrient losses in both protein and calcium were observed when comparing theoretical and laboratory analysis. This aligns with the findings of Ardhanareswari (2019), which demonstrated that laboratory-based nutritional analysis methods have a higher degree of accuracy than theoretical analysis relying on TKPI (2019) and other literature sources[39]. In addition to accuracy levels, factors such as frying temperature can contribute to nutrient denaturation, leading to molecular structural changes and the formation of smaller chains.

Table 5. Protein and Calcium Level Fulfillment of Garlic Stick per Servings Compared to The Indonesian Dietary Recommendation (AKG) 2019

Nutrient	Age (Year)	AKG	Snack Fulfillment Percentage (%)	Nutrient Content (g)		AKG Fulfillment (%)
				Laboratory (100 g)	Nutrient per portion (55 g)	
Protein (g)	4-6	25	10-15	8.26	4.54	18.17%⁺
	7-9	40				11.35%⁺
Calcium (mg)	4-6	1000		89.24	49.08	4.90%
	7-9					

AKG (Indonesian Dietary Recommendation), ⁺) Meeting the required percentage according to AKG

Despite the observed nutrient losses, when comparing theoretical and laboratory analysis results, protein levels in F3 met the snack category requirement for ASD children, (11-18% of daily intake). However, calcium content fulfilled only 4.90% of daily needs per serving, suggesting that consuming two servings would be necessary to meet calcium requirements.

Due to funding, constraints, laboratory nutrient analysis was limited to protein and calcium measurements, and testing was restricted to the control formula (F0) and optimum formula (F3). While the garlic stick could not be categorized as a “source” or “high” in protein and calcium, this study provides valuable insights into the formulation of alternative snacks using local ingredients for ASD children.

CONCLUSIONS

The garlic stick formula containing 175 g MOCAF, 125 g ISP, 30 g medan anchovy flour, and 15 g guar gum was found to be a suitable alternative snack for ASD children. This was supported by hedonic test results indicating favorable acceptability in terms of color, texture, aroma, and flavor, as well as laboratory analysis confirming that it met 11-18% of protein and 4.90% of calcium requirements per serving. To fulfill calcium needs, consumption frequency can be increased to two servings per day.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

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AUTHOR CONTRIBUTIONS

NATA: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, visualization, writing-original draft, writing-review & editing; ACA: conceptualization, formal analysis, methodology, supervision, validation, writing-review & editing.

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