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Synergistic effect of *Chenopodium quinoa* supplementation on the structural, thermal, and nutritional characteristics of gluten-free corn cookies

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Abstract
A research study aimed at developing gluten-free (GF) cookies made from corn and incorporating carboxy methyl cellulose (CMC) alongside quinoa (*Chenopodium quinoa*) was conducted. Employing a completely randomized design, the study featured three independent variables: corn flour, quinoa flour, and CMC. Twelve treatment combinations were created, varying the ratio of corn flour to quinoa flour (100:0, 80:20, 60:40) and incorporating CMC at percentages of 0.25%, 0.50%, 0.75%, and 1.0%. The physical attributes of the resulting cookies, including density, spread ratio, puffiness, width, thickness, and volume index, were assessed by manipulating the levels of corn flour, CMC, and quinoa flour. Statistically significant effects were observed for all three independent variables, with quinoa flour exhibiting a more pronounced impact on nutritional quality and sensory attributes as compared to the other two factors. Numerical optimization led to the identification of the optimal ingredient ratio for formulating cookies as 60:40 for corn and quinoa flour, with a CMC concentration of 0.75%. Consequently, this study affirms the feasibility of incorporating 40% quinoa into corn-quinoa cookie production, providing valuable insights for gluten-free cookie development.

Keywords: Celiac, CMC, CORN, gluten-free, DSC, FTIR, quinoa

Introduction
Gluten, a protein inherent in nature, possesses the capacity to trigger allergic reactions. The escalating occurrence of allergic conditions like celiac sprue and non-gluten wheat sensitivity (NGWS) is propelling a growing worldwide need for products devoid of gluten. (Olawoye *et al*., 2017) [31]. The food industry has recognized the potential of gluten-free foods for daily consumption, spanning a diverse range of products such as bakery items, extruded products, soups, sauces, sausages, and convenience foods. Recent times have witnessed an increased awareness of food intolerance and allergies. It is imperative to incorporate gluten-free (GF) grains in the innovation of food products to ensure a secure and diverse array of gluten-free meals. This, in turn, will contribute to an elevated quality of life for individuals who are intolerant to gluten. Presently, celiac disease affects 1.4% of the global population, with variations between 1.3% in North America and 1.8% in Asia (Lebwohl and Rubio, 2021) [24], while the prevalence of NCG is currently between 0.5 and 13% worldwide (Molina *et al.*, 2015) [29]. Celiac disease (CD) is an autoimmune condition characterized by inflammation of the mucosa in the small intestine, triggered by the intake of gluten and related prolamins found in rye, barley, wheat, and packaged food products.

Quinoa emerges as an exceptional health choice, showcasing an impressive nutritional profile. Rich in essential elements like protein, fiber, vitamins, and minerals, it not only supports muscle growth but also improves digestion and contributes to overall well-being. Furthermore, its gluten-free nature makes it an excellent option for individuals with dietary restrictions. Quinoa and corn are both highly nutritious, offering abundant fiber and serving as a reliable source of essential amino acids, minerals, and proteins. Devoid of gluten, they possess unique attributes such as hypoallergenic properties, a mild taste, and easy digestibility, making them ideal for the progression of baked goods, especially for individuals dealing with celiac sprue. (Mir *et al*., 2017) [28]. However, being GF, they possess low functional qualities.

An amalgamation of hydrocolloids like xanthan gum, gum Arabic, and cellulose derivatives like hydroxypropyl methylcellulose (HPMC) and carboxy methyl cellulose (CMC) are intensively used for enhancing their functional attributes such as their capacity to improve dough viscosity and volume however minimizing the structural hardness and prevent staling. These qualities are typically examined along with sensory qualities and customer approval. Hydrocolloids have been employed in many studies for the development of GF-baked products due to their capacity to alter the rheology and enhance food's sensory appeal. Consequently, hydrocolloids are crucial food additives in the GF baking business (Kamal *et al*., 2015) [16].

Quinoa has captured attention in both research and the creation of various functional products due to its rich nutritional content and exceptional balance of micronutrients and essential amino acids. It stands among the select few plants that offer all the amino acids necessary for human well-being. The quality of its proteins is regarded as outstanding, closely aligning with the ideal amino acid balance set by the FAO, particularly due to elevated levels of lysine and thionic amino acids. The bioavailability of amino acids or protein digestibility significantly increases upon cooking. It exhibits remarkable medicinal and nutraceutical properties, making it a valuable addition to a healthy diet. Packed with essential nutrients, antioxidants, and bioactive compounds, quinoa supports overall well-being. Its potential anti-inflammatory, anti-diabetic, and cholesterol-lowering effects contribute to its reputation as a nutritious and medicinal superfood (Kohajdova *et al*., 2013) [19-20]. The texture of cookies primarily relies on starch gelatinization. With a widespread appeal, cookies serve as vital energy sources for individuals of all ages (Hamdanmi *et al*., 2020) [14]. Their advantages encompass a variety of flavors, adding to their appeal and versatility. Therefore, incorporating quinoa into the production of gluten-free cookies presents an opportunity to craft functional cookies with abundant nutritional and physical attributes.

The present research aimed to craft cookies by incorporating quinoa flour as a protein supplement to corn flour. Formulations were created with the inclusion of plant-based CMC to explore its synergistic impact on the ultimate product's quality.

Materials and Methods

Raw Material

A local landrace of corn (*Zea mays*) was procured from the district Bandipora, Kashmir, cleaned dried, and then milled in a roller mill (Tencan Lab Jar mill) for the development of corn flour. Quinoa flour, baking powder, sugar, butter, vanilla essence, and eggs were purchased from the local market. The cookies were prepared by the creaming method.

Procedure for the formulation of GF corn-quinoa cookies

To create the cookies, quinoa flour replaced a portion of the corn flour within the 20-40% range. Concurrently, carboxy methyl cellulose (CMC) was added at ratios of 0.25%, 0.50%, 0.75%, and 1.0%, as outlined in Table 1. These specified treatment combinations resulted in the preparation of twelve distinct cookie variations at the baking line in the Division of Food Science and Technology, SKUAST, Kashmir, Shalimar Campus, India.

Creating an emulsion involved combining liquid ingredients in a planetary mixer to form a cream, while dry components were gradually incorporated. Carboxy methyl cellulose (CMC) was thoroughly mixed in 80 mL of egg before being added to the dough preparation. The dough underwent blending at 400 rotations per minute for 5-6 minutes until it reached a non-sticky consistency. Following a 20-minute resting period, the dough was frozen at -20 °C for 24 hours. This freezing process facilitates the recrystallization and retrogradation of starch in cookies, contributing to a reduced glycemic index. To attain optimal texture, carboxy methyl cellulose (CMC) was utilized, elevating the texture and thereby mitigating potential problems associated with frozen dough. (Sharadanant and Khan, 2003) [36].

Table 1: Details of treatment combination for the development of Carboxy methyl cellulose incorporated gluten-free corn quinoa cookies.

S. No.	Treatment code	Corn flour (%)	Quinoa flour (%)	CMC (%)
1.	C1Q0H1	100	0	0.25
2.	C1Q0H2	100	0	0.50
3.	C1Q0H3	100	0	0.75
4.	C1Q0H4	100	0	1.00
5.	C2Q1H1	80	20	0.25
6.	C2Q1H2	80	20	0.50
7.	C2Q1H3	80	20	0.75
8.	C2Q1H4	80	20	1.00
9.	C3Q2H1	60	40	0.25
10.	C3Q2H2	60	40	0.50
11.	C3Q2H3	60	40	0.75
12.	C3Q2H4	60	40	1.00

The chilled dough underwent sheeting, cutting, and baking in a BEKO series baking oven at 180 °C (base temperature) and 170 °C (upper plate temperature) for 30 minutes. Following a 10-minute cooling period at 10 °C, the cookies were carefully packaged in aluminum laminated material and stored at room temperature. (19±2 °C) (Mir *et al*., 2017) [28].

Process optimization and validation

The desirability function approach was used to optimize the process. To acquire optimal values for the answers, desired goals were assigned to all of the parameters. Cookies with the highest spread ratio, moderate crispness, and highest overall acceptability were considered best.

Physicochemical composition

Using AOAC (2005) [6] standard procedures, the corn flour and quinoa flour used to make cookies were examined for moisture, crude fat, crude protein, crude fiber, and ash content.

Water activity

A pre-aqua lab, Water activity analyzer was used to determine the water activity of each replicate sample.

Falling number (sec)

The distilled water in the water bath was heated to a boil to determine the activity of -amylase. A viscometer tube was filled with 7 g of flour that had been weighed and transferred. At 20 °C, 25 mL distilled water was added. To achieve a homogeneous suspension, the tube was rubberized and vigorously shaken 30 times. Automatic stirring began after 5 seconds at a rate of 2stirs/sec. After 60 seconds, the stirring ceased automatically, and the time it took the plunger to fall through a particular height was recorded as the falling number. (Zarzycki and Sobota, 2015) [41].

Alkali Water Retention Capacity (%)
The alkaline water retention capacity (AWRC%) was assessed following the procedure outlined in AACC 56–10 (1986) [1]. One gram of flour was immersed in 5 ml of 8.4 g/l NaHCO3 for 20 minutes, followed by centrifugation at 1000 g for 15 minutes at room temperature. The AWRC was determined by weighing the sediment. All results underwent triple verification.

Solvent Retention Capacity (%)
The AACC 56–11 (2002) [2] method was used to get the solvent retention capacity profile (SRC).

Determination of physical attributes
Cookie's width and thickness were measured using a Vernier caliper which has a 0.001-mm precision.
With the following formulas, puffiness and BWL were calculated:

$$\text{Puffiness (\%)} = \frac{\text{thickness of baked cracker} - \text{thickness of unbaked dough}}{\text{thickness of unbaked dough}} \times 100$$

$$\text{BWL (\%)} = \frac{\text{weight of unbaked cookie} - \text{weight of baked cookie}}{\text{weight of unbaked cookie}} \times 100$$

By dividing the mass of the baked cookies made from 100 g of dough mold, the yield % was calculated.

Thermal properties
The thermal characteristics of corn and quinoa flour samples were evaluated using a differential scanning calorimeter, model DSC-822 (Griefensee, Mettler Toledo, Switzerland), equipped with a refrigerator-cooler. Moistened samples weighing 25 grams were airtight sealed in 120 mL aluminum DSC pans. The analysis involved scanning from 20 to 120 degrees Celsius at a rate of 10 degrees Celsius per minute, with a sealed empty pan serving as a reference. Nitrogen was used as the purging gas. Stare software (ver. 9.20, Mettler Toledo) was employed to analyze the generated thermograms. Parameters measured included peak temperature (Tp), end-set temperature (Te), enthalpy change (ΔH) during gelatinization transitions, and onset temperature (To). Gelatinization enthalpy (Hg) based on the primary endothermic peak region was calculated using the Universal Analysis Program. Triplicate analyses were conducted.

$$\text{Degree of gelatinization (DG\%)}: \frac{\Delta\text{H untreated} \frac{\text{corn}}{\text{quinoa}} \text{flour} - \Delta\text{H treated} \frac{\text{corn}}{\text{quinoa}} \text{flour}}{\Delta\text{H untreated} \frac{\text{corn}}{\text{quinoa}} \text{flour}}$$

Since the endotherms are symmetrical, the following equations were used to calculate the total gelatinization temperature range (R) and peak height index (PHI) (Fernandes *et al.*, 2013) [12].

$$R = [2\,(Tp - To)]$$

$$PHI = \frac{\Delta H}{Tp - To}$$

Structural properties
FTIR analysis was used to determine structural characteristics. Mir *et al.*, 2021 [27] methodologies were used to conduct the FT-IR analysis (2015). Before forming a thin pellet, 2 mg of properly dried sample was mixed with 50 mg of desiccated potassium bromide powder. An FTIR spectrometer was used to collect the sample's infrared absorption spectra (Thermo Nicolet 6700, Thermo Scientific, UK) with a resolution of 0.1 cm^{-1} and a scanning range of 4000-450 cm^{-1}.

Amino acid profiling
Utilizing cation exchange chromatography and method 982.30, amino acid analysis was carried out after the protein had been acid hydrolyzed (AOAC, 1990) [6].

Mineral profiling
The vital minerals in the corn and quinoa flours were measured using an atomic absorption spectrophotometer (AAS) made by Hitachi, model Z-5300 (Hitachi Ltd., Hitachi Naka, Japan). According to AOAC method 2.127 (AOAC, 1990) [6] with a small modification, lanthanum chloride was added to the dilution wet-ashes sample solutions during the detection of minerals.

Vitamin profiling
The determination of vitamin content in corn and quinoa flour was carried out using the 1260 Agilent Infinite HPLC Series (Agilent, USA) equipped with a Kinetex-XB-C18 column measuring 100 × 4.6 mm (Phenomenex, USA) and a quaternary pump set at 35 °C. The 1260 separation utilized a binary linear elution gradient consisting of (A) methanol and (B) 25 mM NaH_2PO_4 (pH 2.5, v/v). A 20 µL injection volume was employed, and the VWD detector measured vitamins B3, B6, and B12 at 220 nm, while ascorbic acid was measured at 254 nm.

Instrumental texture profile analysis
Cookie crispness was performed using a TA-HD Plus texture analyzer with a 5-kg load cell (Perkin Elmer Private Limited, UK). The test speed was 1mms^{-1} before the test, 10 mms^{-1} after the test, and 3mms^{-1} during the test. The trigger force was 50 g, and the probe travel distance was kept at 5 mm. The compression plunger was adjusted until it was barely touching the cracker surface in the middle of the sample. The texture was checked. The sample was crushed to a predetermined level (% of compression) by lowering the plunger at a steady speed.

Sensory evaluation
Corn and quinoa flour-based cookies were tested using 20 semi-trained panelists drawn from the SKUAST

community. The cookies were rated for color, appearance, aroma, taste, texture, and overall acceptability on a 5-point scale from excellent (5) to poor (1).

Statistical analysis

Complete randomized design (CRD) was used to figure out which factors were significantly different and were statistically significant with a p of < 0.05.

Result and Discussion

Proximate composition of corn and quinoa flour

The outcomes of assessing the proximate composition of corn flour and quinoa flour, encompassing parameters such as moisture content, crude protein, crude fat, crude fiber, ash, carbohydrates, falling number, viscosity, alkali water retention capacity, water activity, and solvent retention capacity, are detailed in Table 2.

The average water content of corn flour was 9.5% ($p \leq 0.05$), carbohydrates 66.83% ($p \leq 0.01$), crude protein 11.30% ($p \leq 0.05$), crude fat 8.20% ($p \leq 0.01$), crude fibre 2.57% ($p \leq 0.05$), ash 1.6% ($p \leq 0.01$), water activity 0.498 ($p \leq 0.05$), alkali water retention capacity 132.93% ($p \leq 0.01$), solvent retention capacity 78.86% ($p \leq 0.01$), falling number 263sec ($p \leq 0.01$) and viscosity 3261.02 cp ($p \leq 0.01$) while as the average moisture content of quinoa flour was 11.9% ($p \leq 0.05$), crude protein 13.68% ($p \leq 0.05$), crude fat 5.43% ($p \leq 0.01$),crude fibre 5.23% ($p \leq 0.05$), ash 2.2% ($p \leq 0.01$), carbohydrates 61.56% ($p \leq 0.01$),water activity 0.511 ($p \leq 0.05$), alkali water retention capacity 157.96% ($p \leq 0.01$), solvent retention capacity 61.43% ($p \leq 0.01$), falling number 208 sec ($p \leq 0.01$) and viscosity 1126.08 cp ($p \leq 0.01$) respectively.

A notable difference was observed in the proximate composition of the flours ($p<0.05$). Quinoa flour exhibited elevated levels of moisture content (11.9%), crude protein (13.68%), crude fiber (5.23%), water activity (0.511), and alkali water retention capacity (157.96%), whereas corn flour recorded the highest values for crude fat, solvent retention capacity, falling number, viscosity, and carbohydrates. In this study, brown rice flour was found to be indistinguishable from the findings of Rosniyana *et al.* (2011) [34-35] and Islam *et al.* (2012) [15]. The high amount of moisture content in quinoa flour may be attributed to higher protein content having a higher affinity for moisture (Abras *et al.*, 2014) [18]. Quinoa stands out as a superior protein source, possessing higher quality protein compared to wheat, barley, and soybeans, and exhibiting a well-balanced amino acid profile. (FAO, 2011) [1].

Table 2: Proximate composition of corn flour and quinoa flour

Parameter (%)	Corn flour	Quinoa flour	t cal.	p-value
Moisture content	9.5^b	11.9^a	3.4	< .05
Crude protein	11.30^b	13.68^a	6.7	< .05
Crude fat	8.20^a	5.43^b	8.1	< .01
Crude fibre	2.57^b	5.23^a	4.5	< .05
Ash	1.6^b	2.2^a	7.5	< .01
Carbohydrates	66.83^a	61.56^b	8.3	< .01
Water activity	0.498^b	0.511^a	3.1	< .05
Alkali water retention capacity	132.93^b	157.96^a	8.9	< .01
Solvent retention capacity (mm^3)	78.86^a	61.43^b	7.2	< .01
Viscosity (cp)	3261.02^a	1126.08^b	9.3	< .01
Falling number (sec)	263^a	208^b	6.1	< .01

Functional properties of corn flour

In the fabrication of baked goods, the functional qualities of flour are crucial. The functional qualities of the quinoa flour and corn flour are depicted in Table-3. The water absorption index of corn flour (3.52 g/g) was determined to be higher and significant ($p \leq 0.05$) than that of quinoa flour (2.37 g/g). The volume that the starch polymer occupies after expanding excessively in water is determined by the water absorption index. (Anderson *et al*., 1969) [5]. The elevated Water Absorption Index (WAI) in corn flour can be attributed to its higher content of fat and carbohydrates, particularly starch. (Kumar *et al*., 2017) [23]. The water solubility index of corn flour (5.11%) was found to be significantly ($p \leq 0.05$) higher than that of quinoa flour (4.70%). The water solubility index determines the number of free polysaccharides or polysaccharides from starch granules on the addition of excess (Anderson *et al*., 1969) [5]. The highest WSI of corn flour may be attributed to the presence of a higher amount of carbohydrates (starch) (Chandra *et al*., 2013) [9]. The solubility and swelling capacity demonstrate interactions between starch strands and water molecules in crystalline and amorphous areas. The swelling capacities of corn flour and quinoa flour were 5.91 and 6.31 respectively. A significant difference ($p \leq 0.05$) was observed in the solubility capacity of corn flour and quinoa flour. These differences in solubility and swelling capacity may be attributed to the differences in amylose content and viscosity patterns. The primary reported cause of the swelling behavior of grain starches is their amylopectin concentration. Amylose acts as an inhibitor of swelling, especially in the presence of lipids (Ali *et al*., 2016) [4]. Since corn flour contain more starch (amylose) and fat content, therefore, its swelling capacity is less than that of quinoa flour. same findings were previously documented by Chandra and Shmasher (2013) [9] and Islam *et al.* (2012)] [15]. Oil absorption capacity is the ability of a lipid to absorb oil resulting from an interaction with an amino acid non-polar side chain, which gives the product richness and flavors. The Oil absorption capacity of corn flour (CF) and quinoa flour (QF) was 140.49% and 104.63% respectively. A significant difference ($p \leq 0.05$) was observed in the oil absorption capacity of corn flour and quinoa flour. These differences in oil absorption capacity may be attributed to the differences in protein and fat content. The high OAC may be due to an increase in protein which enhanced hydrophobicity by exposing more of a polar amino acid to the fat (Chau & Cheung, 1998) [10].

Table 3: Functional properties of corn flour and quinoa flour

Parameter	Corn flour	Quinoa flour	t cal.	p-value
Water Absorption Index (g/g)	3.52[a]	2.37[b]	3.2	< .05
Water Solubility Index (%)	5.11[a]	4.70[b]	4.8	< .05
Solubility capacity (%)	5.63[b]	11.05[a]	8.1	< .01
Swelling capacity (%)	5.91[b]	6.31[a]	5.7	< .05
Oil Absorption Capacity (%)	113.46[a]	104.63[b]	9.3	< .01

Thermal properties of corn and quinoa flour (DSC)

Gelatinization transition temperatures represent the beginning and end of structural disintegration throughout the gelatinization process The gelatinization onset temperature (T_o), gelatinization peak temperature (T_p), and gelatinization conclusion temperature (T_c) recorded in corn flour was 68.90 °C, 75.68 °C, and 81.18 °C which were in cognizance with the study conducted by Mendez-Montealvo *et al.*, (2006) [26]. However, the gelatinization onset (T_o), gelatinization peak (T_p), and gelatinization conclusion (T_c) temperatures recorded in quinoa flour were 66.10 °C, 73.20 °C, and 79.70 °C. According to Sun et al. (2014) [42], a greater gelatinization temperature range may be attributed to the inclusion of non-starch elements, particularly protein molecules, that impede the gelatinization process. Endotherms were found at considerably more extreme temperature ranges since the moisture content of the flour samples was 11.9% and 9.5%, respectively (Table 2). Quinoa flour had lower To, Tp, Tc, and ΔH values than maize flour. Gelatinization enthalpy (ΔH_{gel}) recorded in the present study suggests that there was a wide difference in heat requirement for gelatinization of different flours. Higher values of ΔH_{gel} is an indicative of heat tolerance (Kraithong *et al.*, 2018) [22].

Table 4: Thermal properties of corn flour and quinoa flour.

Temperature	Corn	Quinoa	t cal.	P value
T_o (°C) Onset temperature	68.90[a]±1.01	66.10[b]±0.86	4.3	< .05
T_P (°C) Peak temperature	75.68[a]±1.00	73.20[b]±0.98	3.2	< .05
T_c (°C) End temperature	81.18[a]±1.12	79.70[b]±1.01	5.1	< .05
ΔH (KJ/Kg) Enthalpy	14.7[a]±0.91	10.90[b]±0.92	7.4	< .01

Structural properties of corn and quinoa flour (FT-IR)

The distinction between maize and quinoa flour was determined through FT-IR analysis, assuming molecularity. The primary difference lies in protein content. Figure 1 clearly illustrates three peaks at wavenumbers 1743.65 cm^{-1}, 1643.35 cm^{-1}, and 1544.98 cm^{-1}, representing Amide I (-C=O) stretching, Amide II (-NH bending and -CN stretching), respectively. Pure maize flour prominently displays the peak at 1743.65 cm^{-1}, with the remaining two peaks observed as strong, sharp features at 1643.35 cm^{-1} and 1544.98 cm^{-1}. Quinoa flour's FTIR spectrum exhibits peaks at 1474.94 cm^{-1} and 1569.97 cm^{-1}, corresponding to amide-I and amide-II, respectively. These distinctive bands, reflecting vibrating patterns for amino acid chains in protein structures, are particularly noteworthy for indicating alterations in secondary protein structural variants. Quinoa flour demonstrates variations in content between 1000 and 1250 cm^{-1} (vibrational modes of C-N and S=O groups), attributed to saponin or sulfate groups linked to the molecular composition of its amino acids. (Garcia-salcedo *et al.*, 2018) [13].

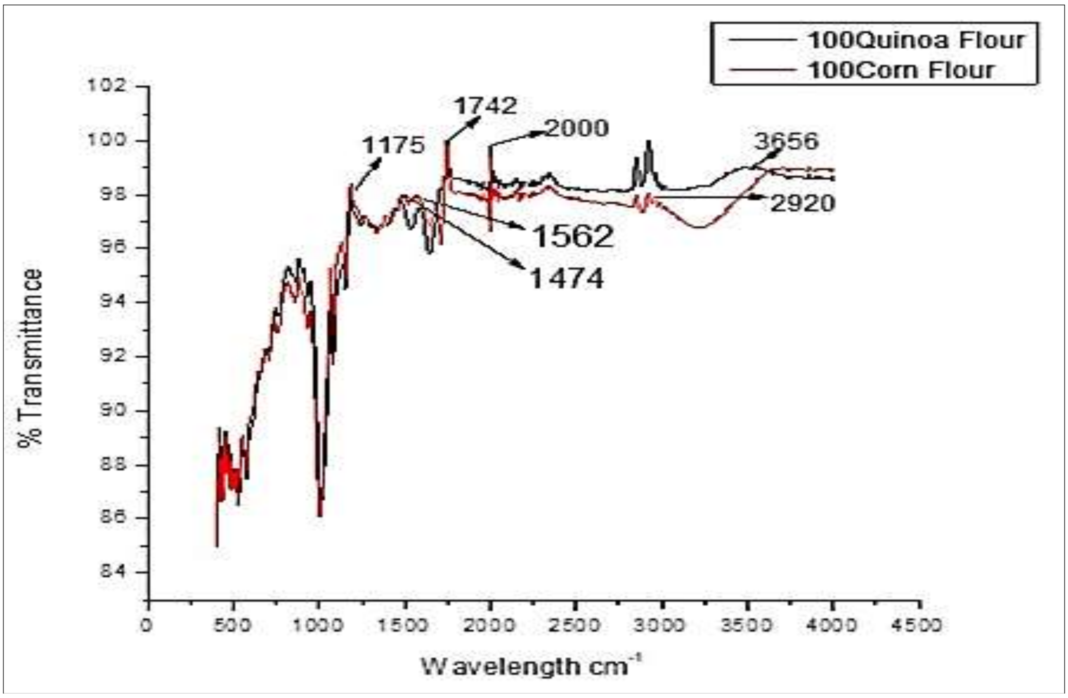


Fig 1: Fourier transform infrared spectrum of corn flour including quinoa flour

Mineral, vitamin, and amino acid profiling.

Amino acid profiling of corn and quinoa flour

Figure 2a illustrates the amino acid composition of maize flour, highlighting elevated concentrations of threonine, phenylalanine, histidine, and arginine. In contrast, quinoa flour stands out for its high levels of lysine (6.33 g/100 g), glutamic acid (0.128 g/100 g), and aspartic acid (0.160 g/100 g), accompanied by notably low proline (0.001 g/100 g) and arginine (9.51 g/100 g) content. Additionally, quinoa flour exhibits moderate levels of glycine (0.096 g/100 g), alanine (5.34 g/100 g), and phenylalanine (4.5 g/100 g). The results suggest that quinoa flour provides substantial concentrations of essential amino acids crucial for human nutrition, including threonine (4.32 g/100 g), alanine (0.53 g/100 g), valine (5.02 g/100 g), methionine (0.80 g/100 g), isoleucine (4.1 g/100 g), leucine (7.4 g/100 g), phenylalanine (4.58 g/100 g), and histidine (3.79 g/100 g). Methionine and isoleucine emerge as limiting amino acids due to their lowest chemical scores. Notably, quinoa flour surpasses wheat protein with its lysine content (6.33 g/100 g), which exceeds that of wheat protein (2.6 g/100 g) (Repo-Carrasco *et al*., 2003) [33]. Earlier studies have observed that quinoa exhibits elevated levels of lysine. (Ranhotra, 1993). With the exception of quinoa, most grains lack sufficient quantities of the crucial amino acid lysine, and only a limited number of legumes provide ample sulfur-containing amino acids such as methionine and cysteine. (Koziol, 1992) [21]. Our findings corroborated with those of (Abugoch *et al.*, 2008) [3] who claimed that quinoa has essential amino acid levels comparable to those of soybean and a similar or high level of histidine (Ogungbenle *et al*., 2009) [30] who claimed that quinoa has balanced essential amino acid levels superior to those of most cereals, such as maize, millet, and sorghum. Hence quinoa can make a great protein supplement.

Vitamin profiling of corn and quinoa flour

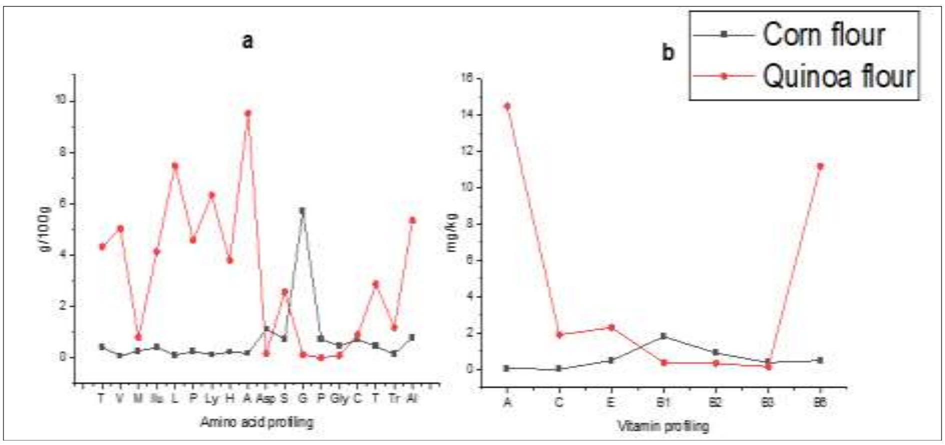
Vitamins are crucial micronutrients required in small quantities to participate in various metabolisms that govern the functions of all living organisms. In Figure 2b, it is illustrated that maize flour contains 0.01 mg/kg of vitamin C, and the niacin content is measured at 0.367 mg/kg. Niacin plays a vital role in overall well-being, contributing to cholesterol regulation and reducing risk factors associated with vascular diseases. The presence of pyridoxine (B6) is noted at 0.48 mg/kg, a vitamin utilized for both preventing and treating anemia. Additionally, pyridoxine is employed in the treatment of high cholesterol, coronary artery disease, and to reduce folate levels in the bloodstream.

Moreover, corn flour exhibits a substantial amount of tocopherols (vitamin E) at 0.49 mg/kg. As depicted in Figure 2b, quinoa flour contains ascorbic acid with a concentration of 1.90 mg/kg, covering 2.2% of the daily human requirements. This underscores the efficacy of quinoa flour in treating and preventing scurvy and cold infections. Additionally, maize flour is noted for its elevated level of alpha-tocopherols (vitamin E), measured at 0.49 mg/kg. The same figure highlights that quinoa flour contains vitamin C at a level of 1.90 mg/kg, meeting 2.2% of daily human nutritional needs. This underscores the potential of quinoa flour in addressing and preventing cirrhosis and viral illnesses.

In the study conducted by Koziol *et al*. in 1992 [21], the observed Niacin content was 0.15 mg/kg, accounting for 10% of the recommended daily intake. This nutrient is crucial for overall well-being, contributing to the improvement of blood cholesterol levels and the reduction of cardiac risks. Additionally, the study found that Pyridoxine (B6) was measured at 11.20 mg/kg, equivalent to approximately 810% of the daily requirements for addressing anemia. Notably, Pyridoxine is utilized in the treatment of cardiovascular heart disease. The results indicate that quinoa flour surpasses main traditional grains such as wheat, barley, and rice in terms of vitamin content. Furthermore, the reports given by (Vega *et al*., 2010) [39], quinoa flour has a high concentration of Vitamin E (2.30 mg/kg), which boosts its anti-inflammatory capabilities by safeguarding the omega-3 fatty acids in cell walls from damaging free radicals.

Mineral profiling of corn and quinoa flour

Figure 2c illustrates the mineral contents of corn and quinoa flour. There was a significant increase ($p<0.05$) in the mineral percentage in maize flour. Consistent with previous literature, the quantitative analysis of maize flour revealed lower sodium levels (7.08 mg/kg) and higher levels of potassium (779.11 mg/kg), magnesium (93.09 mg/kg), zinc (2.67 mg/kg), phosphorus (242.83 mg/kg), calcium (132.45 mg/kg), and iron (1.44 mg/kg). Consequently, these mineral components may play a role in regulating cellular water balance for metabolic processes. For instance, potassium aids in mitigating high blood pressure, while the calcium levels found in composite wheat are adequate for bone and dental development. Inadequate intake of zinc and iron has been associated with severe anemia, exacerbated illnesses, and mental health issues. (FAO, 2001) [11]. The provided data illustrates that quinoa flour exhibits elevated levels of sodium (854.22 mg/kg), potassium (443.14 mg/kg), magnesium (173.31 mg/kg), and calcium (864.89 mg/kg), with a moderate concentration of iron (73.07 mg/kg) and zinc (25.40 mg/kg).



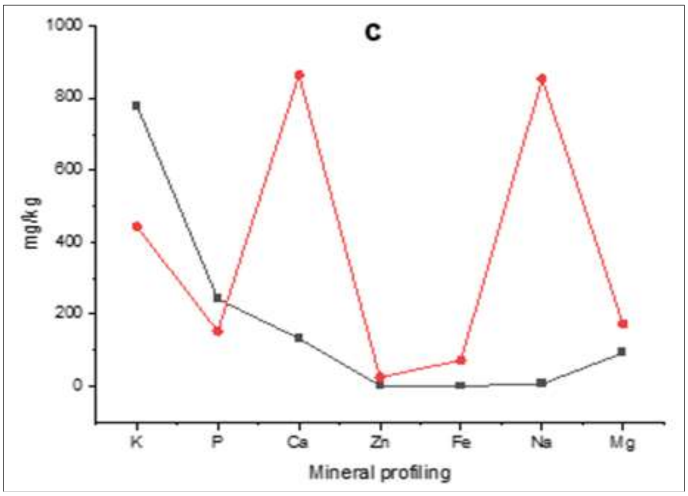


Fig 2(a): Amino acid profiling * (b) Vitamin profiling** (c) Mineral profiling***

Our findings revealed that quinoa flour possesses an iron content 6.5 times greater than that of wheat, underscoring its efficacy in addressing deficiencies. With an average concentration of 443.14 mg/kg, potassium emerged as the mineral with the highest concentration, following closely behind sodium, which measured 854.22 mg/kg. It is widely acknowledged that quinoa flour tends to have elevated levels of potassium and sodium when the cereal crop is cultivated in arid or saline conditions. Given that minerals such as potassium, sodium, and magnesium function as neurotransmitters crucial for bodily processes, their presence is vital. However, a calcium deficiency can pose challenges for newborns in forming their oral structures. Previous research has consistently indicated that quinoa seed flour possesses an exceptionally suitable and optimal composition of vital minerals when compared to other major cereals.

Physical parameters of gluten-free corn-quinoa cookies

The physical parameters that were studied after developing the twelve treatment combinations of corn quinoa cookies were, width, thickness, spread ratio, puffiness, bulk density, baking weight loss, volume index, yield, and hardness-Table 3).

The thickness of developed cookie was discovered in the series of 8.22 to 10.57 mm. The thickness of cookies increased with the incorporation of quinoa flour (10.57-10.98 mm) while the breadth of the cookie developed was discovered to be in the range of 43.80 to 46.94 mm (Table 9), and width decreased due to the incorporation of quinoa flour (44.20-43.80 mm). According to Islam *et al.,* (2012) [15], the rise in density might be attributed to a reduction in breadth, as represented in the spread ratio. The particle size and amount of quinoa flour influenced the breadth of the cookies, since bigger quinoa flour particles may have a negative impact on the dimension of the cookies (Bose *et al.* 2010) [7].

Furthermore, with the increase in the concentration of CMC, width increased from 44.20-46.94 mm,44.00-46.66 mm, and 43.80-46.34 mm in case of (100C:0Q:0.25-1%CMC), (80C:20Q:0.25-1%CMC) and (60C:40Q:0.25-1%CMC) while thickness decreased significantly from 10.57-7.93 mm(100C:0Q:0.25-1%CMC),10.75-8.09 mm (80C:20Q:0.25-1%CMC) and 10.98-8.22 mm (60C:40Q:0.25-1%CMC). In addition, Kaur *et al*. (2015) [17] noted that the use of hydrocolloids increased the diameter of cookies made with buckwheat flour. The distribution or spread ratio is regarded as a fundamental criterion for cookies since it is associated with appearance, grain fitness, bite, and general taste experience (Bose *et al.,* 2010) [7]. With the addition of quinoa flour, the final spread ratio fell considerably (P 0.05) (4.18-3.98). The lower spread ratios of quinoa flour-enhanced corn flour cookies may be due to a spike in the number of aquaphobic spots fighting for constrained liberated water in dough (Table 10). The number of hydrophilic sites increases due to the seemingly generated clumps by blended flour (Islam *et al.* 2012) [15].

During dough mixing, these hydrophilic spots rapidly partition their free water, which enhances dough viscosity and limits cookie spread (Kohajdova *et al.,* 2013) [19-20]. The addition of quinoa flour might have diminished the spread ratio (SR) by elevating the protein content and, at the same time, reducing the overall gluten level. Singh *et al.* (2003) [37] also found that the spread ratio (SR) in cookies decreased as the non-wheat protein was raised. At increasing quantities of the CMC, SR rose probably due to lower dough viscosity, resulting in higher SR in the ranges of 4.18-5.91, 4.09-5.76, and 3.98- 5.63 for (100C:0Q:0.25-1%CMC), (80C:20Q:0.25-1%CMC), and (60C:40Q:0.25-1%CMC). Quinoa flour was added to corn cookies, reducing their puffiness (63.58- 61.63). This might be because the lack of the gluten network prevents the quinoa proteins from holding air cells, which reduces puffiness (Islam *et al.* 2012) [15]. However, CMC had a constructive outcome on the proportion of puffiness in cookies. Cookies become puffier because CMC creates hydrophilic side-chained elastic micro-gels surrounded by air microcapsules to prevent the structure from coalescing (Hamdani *et al.,* 2020) [14].

Bulk density (BD) plays a crucial role in determining the acceptability of cookies. The introduction of quinoa flour (0-40%) significantly elevated the general hardness (BD) from 0.80-1.32. This increase is largely attributed to the particle size of the quinoa flour, as BD is closely linked to the dimensions of the flour particles. Given that quinoa flour has a larger granular dimension, the bulk density experiences a noticeable rise with the escalating concentration of quinoa flour (Islam *et al.* 2012) [15]. According to Wongklom *et al.* (2016) [40], CMC incorporation up to a certain level prevents the structural collapse of the bread, increasing volume and lowering density. So, BD decreases with the increase in the level of CMC from 0.80-0.48 g/cc (100C:0Q:0.25-1%CMC),1.00-

0.80 g/cc (80C:20Q:0.25-1%CMC), and 1.32-0.92 g/cc (60C:40Q:0.25-1%CMC) respectively.

Baking weight loss (BWL) is a measure of moisture loss and the concurrent formation of pores during baking (Chevallier *et al*., 2002). The incorporation of quinoa flour resulted in a significant increase in bulk water absorption (BWL), rising from 15.31% to 17.38% ($p \leq 0.05$). This phenomenon is linked to the absence of a gluten network, which causes a reduction in starch polymers within the matrix. As a result, there is a limitation on the excessive swelling of starch granules and the dispersion of components (Olga *et al*., 2021). As the content of CMC increased, there was a decrease in bulk water absorption (BWL), possibly attributable to the high water-holding capacity (WHC) of gums. CMC, being a cellulose derivative, forms hydrogen bonds with a greater number of hydrophilic groups. This transformation results in the conversion of free water into bound water at relatively higher concentrations, thereby restricting moisture loss through evaporation (Maleki and Milani, 2013) [25].

Due to the dual impact of gluten dilution and the mechanical disruption of the gluten network structure caused by quinoa particles, our findings revealed a decrease in the volume index upon the addition of quinoa flour. The presence of diluted gluten reduces the dough's capacity to trap air and retain gas, consequently leading to a reduction in the volume index. (Mohammed *et al.* 2012). Moreover, the introduction of CMC led to a decrease in the volume index of cookies, dropping from 0.504 to 0.305 cm³. This decline can be ascribed to a reduction in the cohesive protein matrix, as well as a decrease in the dough's elasticity and extensibility, ultimately resulting in a diminished cookie volume (Wongklom *et al.* 2016) [40].

The inclusion of quinoa flour resulted in a notable reduction in yield, decreasing from 79.00% to 74.05% ($p \leq 0.05$). This decrease can be attributed to an elevated baking weight loss caused by quinoa flour's pronounced affinity for moisture (Table 3) (Caratini and Rosentrator 2019) [8]. Hydrocolloid usage, according to Mohammadi *et al.* (2014), reduces weight loss while enhancing baking yield. The yield might have increased because CMC typically enhances moisture absorption and lessens significant heating loss. Mohammadi *et al*. (2014) found that as CMC concentration increased, as bread yield in the case of (100C:0Q:0.25-1%CMC), (80C:20Q:0.25-1%CMC), and (60C:40Q:0.25-1%CMC) respectively in the range of 76.12-79%, 75.09-78.18% and 74.05-77.14% respectively.

The crispness in cookies was boosted substantially via 24.309-49.551 (P 0.05) with the inclusion of quinoa flour, the fiber content drastically levels up resulting in higher toughness (Sudha *et al.* 2007) [38], despite other studies relating increased toughness in biscuits and cakes with substantial protein quantities in the composition (Pareyt *et al.* 2011) [32]. Due to the higher protein and fiber content in quinoa flour compared to corn starch, it is conceivable that these variations in protein and fiber levels played a role in the increased hardness observed in the quinoa cookies. According to Wongklom *et al*. (2016) [40], CMC incorporation up to a certain level prevents the structural collapse of the bread, increasing volume and crispness from 24.309-43.497, 26.220-45.314, and 29.187-49.551in the case of (100C:0Q:0.25-1%CMC), (80C:20Q:0.25-1%CMC), and (60C:40Q:0.25-1%CMC) respectively.

Table 3: Physical parameters of gluten-free corn-quinoa cookies.

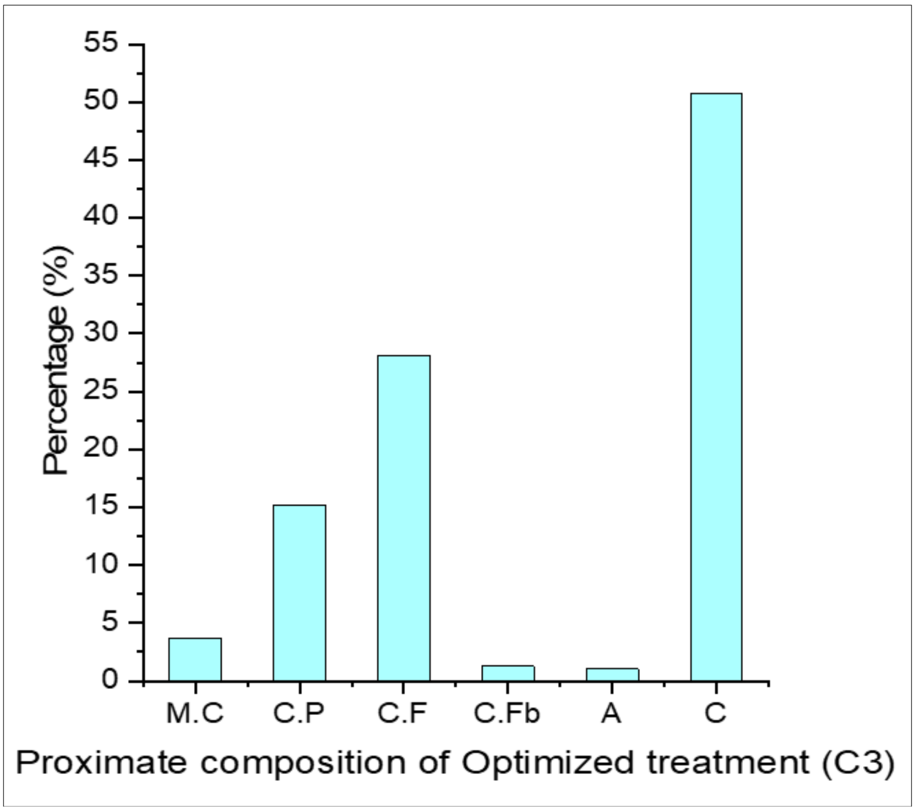
Treatments	Thickness (mm)	Width (mm)	Spread Ratio (mm)	Puffiness (%)	Bulk density (g/cc)	Baking weight loss (%)	Volume index (cm³)	Yield (%)	Hardness (N)
A1	10.57	44.20	4.18	63.58	0.80	15.31	0.504	76.12	24.309
A2	9.55	45.22	4.73	64.60	0.65	14.28	0.402	77.15	26.968
A3	8.53	46.24	5.42	65.63	0.52	13.24	0.308	78.19	37.660
A4	7.93	46.94	5.91	66.13	0.48	12.84	0.227	79.00	43.497
B1	10.75	44.00	4.09	62.56	1.00	16.35	0.409	75.09	26.220
B2	9.72	45.03	4.63	63.59	0.95	15.31	0.357	76.12	31.720
B3	8.69	46.06	5.30	64.61	0.89	14.28	0.305	77.16	38.447
B4	8.09	46.66	5.76	65.21	0.80	13.88	0.274	78.18	45.314
C1	10.98	43.80	3.98	61.63	1.32	17.38	0.305	74.05	29.187
C2	9.95	44.82	4.50	62.65	1.22	16.34	0.283	75.08	35.961
C3	8.92	45.84	5.13	63.68	1.07	15.31	0.264	76.12	41.233
C4	8.08	46.34	5.63	64.38	0.92	14.81	0.242	77.14	49.551
Mean	9.31	45.42	4.93	64.02	0.88	14.94	0.32	76.61	35.83
CD ($p \leq 0.05$)	0.61	0.29	0.15	0.79	0.07	1.03	0.025	1.09	0.693

Optimization

The optimization results indicate that the cookies from treatment C3 (60% corn: 40% quinoa: 0.75% CMC) represent the optimal combination, exhibiting the highest spread ratio, moderate crispness, and the highest overall acceptability.

Proximate composition of Carboxy Methyl Cellulose incorporated gluten-free corn-quinoa cookies.

The moisture content of cookies developed from corn-quinoa was 3.68 ±0.02%. The cookies have a high protein content of 15.16 ±0.06% because quinoa flour has high protein content (Khoshgozaran *et al*., 2014) [18].The fat content increased with the addition of quinoa flour (28.08±0.03). Increased oil absorption increases creaminess and preserves cookie flavor. As a result of the use of quinoa flour, the amount of carbohydrates turned out to be low (50.76 0.09%) in the best cookie (Figure 3a). The significant intake of fiber and mineral count of quinoa flour contributes to the cookie's ash content.



Note: *Moisture content (M.C), crude protein (C. P), crude fat (C. F), crude fiber (C. Fb), ash (A), carbohydrate (C).

Fig 3a: Proximate composition of C3*

Sensory Evaluation (5-point scale)

Figure (3b) depicts the sensory score of the developed cookies of all treatment combinations. The scores for appearance, color, crispness, mouthfeel, and OAA increased with the increase in the concentration of CMC from 0.25 to 1% and also with the rise in the proportion of quinoa flour from 0-40% in the range of 3.67 to 4.15, 3.72 to 4.15 and 3.90 to 4.50 in case of 100% C:0% Q:0.25% to 1% CMC; 80% C:20% Q:0.25% to 1% CMC and 60% C:40% Q:0.25% to 1% CMC respectively.C3 (60:40:0.75%; C:Q:CMC) cookies has the highest OAA than other 11 treatment combinations.

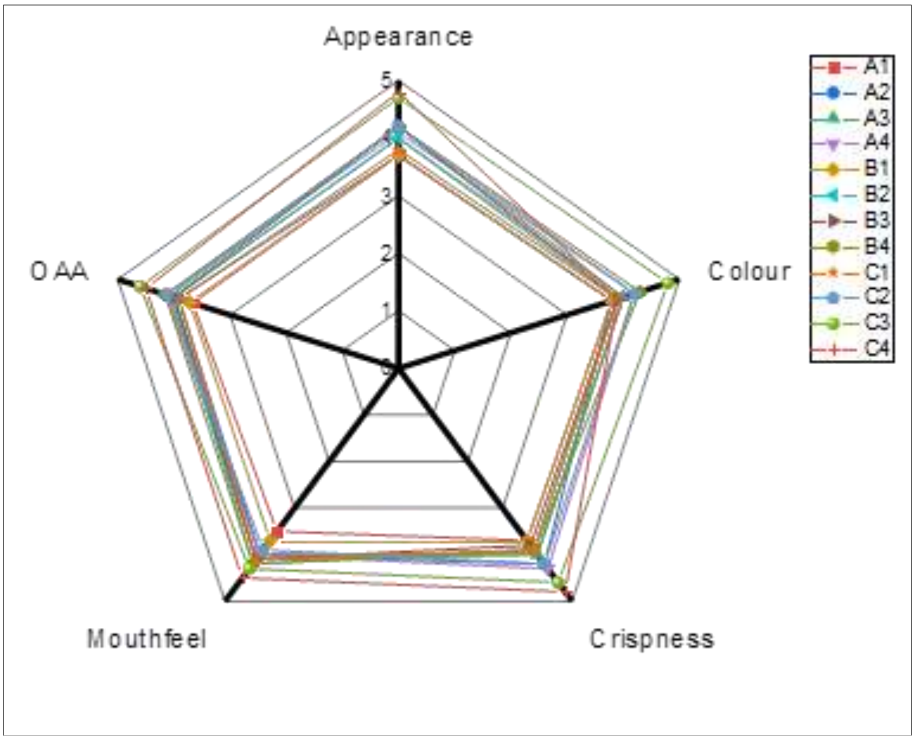


Fig 3b: Sensory Evaluation of the developed corn-quinoa cookies.

Conclusion

To fulfill the consumer demand of using diverse ingredients to produce beneficial and worthwhile functional products its required that the nutritional content of the cookies is profuse apart from being gluten free by nature. Best product was obtained at a composition of 60% corn flour, 40% quinoa flour and 0.75% CMC. The optimized corn-quinoa biscuits demonstrated excellent sensory appeal and acceptable intentions to buy, indicating they're suitable for wide community ingestion. It's also an intriguing alternative for people who have celiac disease. It is well known that gluten-free cookies typically have lesser quality than traditional cookies, yet the inclusion of CMC raised the chemical, physical, textural, and sensory properties of gluten-free cookies.

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