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Evaluation of the effect of quinoa flour replacement on quantitative and qualitative characteristics of gluten-free cookie using principal component analysis method (PCA)

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ABSTRACT

This study aims to investigate the effect of substituting different levels of chickpea flour (0, 25, 50, 75 and 100%) with quinoa flour on the relationship and dependence between the amount of protein, ash, moisture, specific volume, texture, color changes and sensory characteristics. Gluten-free cookies were analyzed using principal component analysis (PCA). The obtained results showed the dependence and relationship between the evaluated parameters to the change in the level of substitution of chickpea flour with quinoa in the formulation of gluten-free cookies. By replacing 25%, the relationship between the parameters changed significantly. With the increase of substitution level change from 25 to 50%, with the predominance of smell and taste and change in texture, the relationships between the evaluated parameters in the space of principal component analysis were rearranged relative to each other. By increasing the substitution level from 50 to 75%, the influence of the presence of quinoa flour on the textural characteristics of the manufactured product and its relationship with other parameters showed the variation of the examined traits. This indicated the critical level of relationship change towards the predominance of quinoa properties in the formulation. At the replacement level of 100%, based on the position of the special volume parameter with the parameters related to tissue characteristics, an opposite relationship was observed between them. Based on the results, it shows the applicability of principal component analysis as a useful tool in identifying the substrate of relationships between quantitative and qualitative parameters in the direction of designing and developing food formulations, and finally, the maximum replacement of 50% of chickpea flour with quinoa flour in Chickpea sweet formulation is recommended.

1. Introduction

Celiac disease, a genetically predisposed autoimmune disorder, profoundly affects the small intestine. This digestive ailment manifests in the deterioration of intestinal villi, compromising the absorption of essential substances. Ingestion of gluten (specifically gliadin), a protein found in wheat, barley, rye, and occasionally oats, triggers symptoms of intolerance in celiac individuals, ranging from mild discomfort to life-threatening complications. To date, no definitive cure or therapeutic intervention exists for celiac disease. Management relies solely on strict adherence to a gluten-free diet, excluding any food containing the offending protein [1]. Unfortunately, commercially available gluten-free products often fall short nutritionally, lacking protein and other crucial nutrients compared to their gluten-containing counterparts [2]. Chickpea sweets, a traditional delicacy in Qazvin province, offer a promising gluten-free alternative [3]. Its components lack gluten, making it suitable for incorporation into the dietary regimen of individuals with celiac disease. Additionally, legumes such as peas provide protein and soluble fiber without the adverse effects of gluten. Quinoa, a gluten-free pseudo-cereal, significantly enhances the nutritional profile of chickpea sweets. Ouinoa, classified as _Chenopodium quinoa Willd_, belongs to the _Chenopodiaceae_ family. Its seeds are exceptionally light and digestible. Rich in protein, quinoa contains essential amino acids (lysine, methionine, cysteine, and threonine), minerals (calcium, phosphorus, magnesium, potassium, iron, copper, manganese, and zinc), dietary fiber, and Bcomplex vitamins [4, 5, 6]. Its nutritional value rivals that of powdered milk, as recognized by the FAO [7]. Incorporating quinoa flour into chickpea sweets formulations can dramatically improve the product's dietary attributes.

Principal Component Analysis (PCA) is a multivariate analysis technique employed to explore the relationships between multiple data layers. This method transforms data sets with numerous dimensions or attributes into a vector space, enhancing the interpretability of data connections [8]. PCA has proven valuable in examining sensory attributes and quantitative parameters, revealing their interdependencies [9, 10, 11]. In the context of food products, sensory characteristics hold particular significance for consumers with dietary restrictions [12, 13, 14]. Due to constraints in using specific ingredients, identifying alternative substitutes is crucial. Understanding the relationship between quantitative and qualitative parameters under varying substitution levels of compounds in food enables informed product formulation [15]. This study utilizes PCA to unravel the associations between quantitative and qualitative aspects of chickpea sweets while replacing chickpea flour with quinoa flour at varying proportions.

2- Materials and methods

2-1- Materials

Quinoa flour with 7.67% moisture, 5.7% fat, 17.92% protein, and 2.84% ash, chickpea flour with 5.23% moisture, 4.1% fat, 24.23% protein, and 63% ash. 2.0%, powdered sugar and shortening were purchased from a reliable confectionery.

2-2-The method of making sweets

The control gluten-free pastry (without quinoa flour) contained 100 grams of chickpea flour, 50 grams of shortening, and 50 grams of powdered sugar. To prepare the treatments, chickpea flour was replaced with quinoa flour at 0, 25, 50, 75, and 100% levels. For this purpose, sugar powder and shortening were first mixed in a laboratory mixer tank (model BJY-BM5N manufactured by Bek Sanat Company, Iran) for 5 minutes until a smooth and uniform mixture was reached. In the next step, flour was added to the mixture of powdered sugar and shortening in several stages, and the mixing continued until a uniform dough was reached. Then the transferred pastry dough was placed in polyethylene bags for 24 hours at 4 degrees Celsius (refrigerator temperature). After the resting period, the pastry dough was removed from the refrigerator and placed in the environment until it reached a temperature of about 25 degrees Celsius. Next, the gluten-free pastry dough was rolled out to a diameter of 1 cm wide and molded with a small four-pin mold. At the end, the prepared samples were placed in a special tray and baked in a laboratory oven (model XFT135, made in Italy) at a temperature of 160 degrees Celsius for 20 minutes.

2-3-Protein

AACC method (2000) No. 46-12 (Kjeldahl method) was used for protein measurement [15]. For this purpose, 1 gram of sample was carefully weighed and placed in a digestion tube. 0.8 g of the catalyst mixture was poured on the sample, then 3 ml of

concentrated sulfuric acid was added to the tubes with a pipette. The tubes were placed on digestion furnaces and heated slowly. When some of the emitted smoke and vapors decreased, the heat increased. During the digestion, the tubes were shaken several times so that, if possible, any substances attached to the body enter the solution. After the contents of the tube became clear and colorless, the digestion continued for about 20 minutes. After the end of digestion, the tubes were cooled, then 25 cc of distilled water was added to each tube and mixed. The tube was placed inside the device that directly measures the amount of protein. Finally, the amount of protein was calculated by adjusting the device parameters such as nitrogen and protein ratio.

2-4-Ash

AACC method (2000) No. 01-08 was used to measure ash [15]. For this purpose, a porcelain plant was completely dried in the oven and then cooled and weighed in a desiccator. 5 grams of the sample was weighed, placed in a porcelain crucible, and placed inside the furnace. After the preliminary burning, the temperature of the furnace was increased to 550 degrees Celsius and the heating continued until the color of the ash became lighter. When the dark color remained, the plant was taken out of the furnace and cooled and a few drops of distilled water were added to it, and after evaporation, it was put back into the furnace until light ash was produced. Finally, the porcelain flask containing the sample was taken out cooled in a desiccator, and then weighed. Equation 1 was used to determine the percentage of ash.

Equation 1: Ash (%) = $((a_1-a_2)/a_0) \times 100$

In this regard, a_0 is the weight of the sample, a_1 is the weight of the porcelain bush and the sample before being placed in the furnace, and a_2 is the weight of the bush and the ash of the sample after being placed in the furnace.

2-5-Moisture

Moisture content was measured using the AACC (2000) method No. 44-16 (oven drying method) [15]. For this purpose, at first, the special plate was placed in the oven at 103 degrees Celsius for at least 0.5 hours, then it was cooled in a desiccator at laboratory temperature and weighed with an accuracy of one milligram. Then, 10 grams of the sample was transferred to a container and weighed

again carefully. Next, the plate containing the sample was placed in an oven with a temperature of 105 degrees Celsius. Then, after about 3 hours, the plate was removed from the oven and after cooling in a desiccator, it was weighed again. The process of weighing the sample continued until a constant weight was reached. The amount of moisture percentage was calculated from Equation 2.Equation 2: MC (%) = $[(m_1-m_2)/m_0] \times 100$

In this regard, m_0 is the weight of the weighed sample, m_1 is the weight of the plate and the sample before being placed in the oven, and m_2 is the weight of the plate and the sample after being placed in the oven.

2-6-Specific volume

For this purpose, the weight of each sample was measured using a digital scale. Then, the volume replacement method with rapeseed was used according to the AACC method (2000) No. 72-10 to measure the volume. Finally, by dividing the volume by the weight of the sample, the specific volume was calculated in milliliters per gram [15].

2-7-Texture

The texture evaluation of the samples was done using a texture measuring device based on the method of Nasahi et al. (2018) with some changes. For this purpose, each treatment (a complete glutenfree pastry) was compressed with a plate probe larger than the cross-sectional area of the sample at a speed of 5 mm/s up to 50% of the thickness of the pastry, and the maximum force required as texture hardness in Newton will be considered [16].

2-8-Color

The color evaluation was done according to the method of Sarikoban and Yilmaz et al. (2010). For this purpose, the samples were placed in a box with a white background for photography, and photography was done using a digital camera. Factors related to color were obtained in the Huntlab system (L*a*b*), by transferring images to a computer and using image processing software. This model has the L* factor (including the black-to-white spectrum with a range from zero to 100) and two color factors a* (including the green-to-red color spectrum with a range of +120 to 120) and b* (including the blue to yellow color spectrum with The range is +120 to -120). After determining the color components to compare the color of the

production samples (different treatments), the color change index (Color) was calculated using Equation 3 [17].

Equation 3: Color =
$$\sqrt{(L-L0)^2 + (a-a0)^2 + (b-b0)^2}$$

 L_0 , a_0 and b_0 are the color components of the control sample.

2-9-Sensory properties

For this purpose, 10 referees were selected. Sensory characteristics of gluten-free sweets in terms of form and shape (asymmetrical shape, and the presence of any cavity or internal space), surface (bluntness, abnormal color, wrinkles, cracks, and abnormal surface), firmness and softness of texture (doughy or unusual softness, firmness, crispness, and fragility), chewability (dryness and firmness, lumpiness and paste in the mouth and sticking to the teeth) and smell, taste, and flavor (pungent taste, smell rawness or sourness or the natural aroma of gluten-free sweets) were evaluated. Attribute evaluation coefficient based on five-point hedonic from very bad (1) to excellent (5) and the ranking coefficient of each of the characteristics (form and shape, surface, firmness and softness of texture, chew ability, and smell and taste) respectively It was 4, 2, 2, 3 and 3 [18 and 19]. Finally, with this information, the overall acceptance score (quality number of gluten-free sweets) was calculated using Equation 4.

Equation 4: $Q = [\Sigma(P \times G) / \Sigma P]$

2-10-Statistical analysis

The present study investigates the impact of incorporating quinoa flour at varying levels (0%, 25%, 50%, 75%, and 100%) on the characteristics of gluten-free chickpea sweets. Using a randomized design, fifteen samples were prepared with three replications per treatment. Principal component analysis (PCA), a multivariate statistical technique, was employed to identify the principal components explore the relationships among the and characteristics of the chickpea sweets. The PCA model was constructed using the first and second principal components, which accounted for a substantial proportion of the information contained within the dataset. The software package Minitab 17 was utilized for the analysis.

3- Results and discussion

3-1- Analysis of relationships between quantitative and qualitative parameters of gluten-free sweets without quinoa flour

Figure 1 visualizes the correlations between quantitative and qualitative parameters of glutenfree sweets devoid of quinoa flour using principal component analysis. The analysis reveals a direct influence of two parameters, chewability and softness, on overall acceptance. This suggests a strong reliance on taste judgments on mouthfeel characteristics. Sensory evaluations of oral and textural attributes encompass a spectrum of characteristics, including mechanical, structural, and rheological properties [20]. Conversely, a dependent relationship exists between qualitative parameters (shape and form) and two quantitative (moisture and ash). parameters Negative correlations were observed between specific pairs of parameters: texture parameters with specific volume, color with moisture, aroma with protein, color with form, and surface parameters with texture. These relationships indicate mutual influences among quantitative and qualitative parameters in chickpea-based sweets. Consumers initially evaluate new food products based on appearance, such as surface characteristics, shape, and form [22, 21]. Thus, the uneven relationship between product surface characteristics and softness and shape and color suggests that consumers effectively use appearance cues to judge sensory qualities. The influence of protein content on perceptual taste characteristics can be analyzed from multiple perspectives. Structurally, proteins consist of amino acid sequences. Denaturation and structural alterations increase the availability of amino acids for interactions with other formulation components. This enhanced interaction transforms sensory properties related to aroma and taste reception [23]. Protein content in the product is derived from chickpea proteins. Increasing chickpea protein concentration results in a sandy/chalky texture, augmented swelling, and an unpleasant swallowing sensation. These effects are attributed to hydrophobic, hydrogen, and van der Waals interactions between protein chains, which impact sensory properties and taste perception [24]. Proteins exhibit an amphiphilic nature (concomitant presence of hydrophilic and hydrophobic groups in the chain). Expansions in hydrophobic interactions lead to protein denaturation and conformational changes [25]. Denaturation enhances structural alignment and availability of amino acids in the

protein chain. Consequently, the presence of sugars in the formulation promotes a heightened Maillard browning reaction, which generates high molecular weight compounds and undesirable flavors in the product [26, 27].



Figure 1- Analysis of relationships between quantitative and qualitative parameters in gluten-free sweets without the substitution of quinoa flour in the principal components analysis space.

3-2- Analysis of relationships between quantitative and qualitative parameters of gluten-free sweets containing 25% quinoa flour

Figure 2 illustrates the correlations between quantitative and qualitative parameters of glutenfree sweets containing 25% quinoa flour within the principal component analysis (PCA) space. The PCA analysis highlights that alterations in formulation impacted the sensory evaluations of taste judges, leading to variations in correlations with quantitative product characteristics, resulting in both consistent and inconsistent relationships. The PCA revealed a strong positive correlation among three parameters including specific volume, moisture content, ash content, and protein content. Substituting 25% chickpea flour with quinoa flour, compared to the control sample without quinoa flour, demonstrated a direct relationship between specific volume, shape, and form with moisture and ash content. Additionally, the presence of 25% quinoa flour exhibited a negative correlation between protein content and consistent relationships between aroma and taste parameters with chewability, shape, and texture. Turkat et al. (2016)

examined the formulation of gluten-free bread incorporating quinoa flour, buckwheat, rice flour, and potato starch. Their findings indicated that the sample containing 25% quinoa flour enhanced the textural properties of the bread [28]. They reported that the incorporation of 25% quinoa flour resulted in a softer texture with desirable sensory characteristics. Jan et al. (2018) utilized quinoa flour in the development of gluten-free cookies. Their research demonstrated that using less than 50% quinoa flour improved the texture of the cookies. They attributed this improvement to the quinoa flour's ability to increase the spreadability of sugar and oil within the formulation, thereby reducing the stiffness of the texture [29]. These reported results align with the sensory observations of parameter correlations in the PCA space for gluten-free sweets enriched with quinoa flour. Notably, the parameter for general acceptability, which serves as a comprehensive measure of sensory characteristics evaluated by taste judges, exhibited a strong positive correlation with surface characteristics and was influenced by chewability, smell, and taste parameters.



Figure 2- Analysis of relationships between quantitative and qualitative parameters in gluten-free sweets with 25% substitution of quinoa flour in the principal components analysis space.

3-3- Analysis of relationships between quantitative and qualitative parameters of gluten-free sweets containing 50% quinoa flour

Multivariate analysis reveals the complex interrelationships between sensory and objective parameters of gluten-free sweets incorporating 50% quinoa flour (Figure 3). Increasing substitution of chickpea flour with quinoa flour induced an inverse relationship between overall acceptance and chewability. Conversely, a direct and consistent relationship emerged between acceptance and both product softness and shape. Odor and taste parameters exhibited a direct correlation with specific volume parameters but an inverse correlation with color. A notable inconsistency was observed between the sensory softness score and the objective texture measurement (hardness), suggesting that quinoa's incorporation significantly altered textural perception. Notably, the substitution of chickpea flour with quinoa from 25% to 50% resulted in a marked shift in the product's sensory balance, characterized by a more pronounced quinoa aroma and taste, accompanied by a distinct change in texture attributable to quinoa's presence. These findings align with Nesliha et al. (2018), who reported an increase in cake volume and a decrease in texture with increasing quinoa flour inclusion in gluten-free formulations [30].



Figure 3- Analysis of relationships between quantitative and qualitative parameters in gluten-free sweets with 50% substitution of quinoa flour in the principal components.

3-4- Analysis of relationships between quantitative and qualitative parameters of gluten-free sweets containing 75% quinoa flour

Figure 4 shows the relationships between quantitative and qualitative parameters of glutenfree sweets containing 75% quinoa flour in the component principal analysis space. The distribution of investigated traits reveals а divergence in relationships between evaluated parameters and a reduction in dependence between aligned and non-aligned dimensions as the substitution of chickpea flour with quinoa flour increases from 50% to 75%. This indicates a critical level of change in relationships, favoring quinoa

flour's dominance in the formulation and creating independent characteristics. Overall acceptance is significantly influenced by the attributes of form and shape and softness and chewability. Conversely, smell and taste parameters demonstrate an opposite relationship with texture. Among evaluated traits at 75% substitution, color parameters, surface area, and specific volume exhibit the strongest correlation in the same direction. The observed relationships at the 75% replacement level suggest a direct influence of quinoa on gluten-free sweets' textural properties. These textural changes directly impact sensory properties, particularly smell and taste perception. The release of aromatic compounds during texture breakdown enhances oral taste perception. Previous

research by Milovanoik et al. (2014) demonstrated an increase in protein, crude fiber, and oil content in wheat flour-quinoa flour bread samples compared to control bread. Consequently, bread core hardness and strength increased linearly with replacement levels exceeding 50% [31].



Figure 4- Analysis of relationships between quantitative and qualitative parameters in gluten-free sweets with 75% substitution of quinoa flour in the principal components.

3-5- Analysis of relationships between quantitative and qualitative parameters of gluten-free sweets containing 100% quinoa flour

Figure 5 illustrates the interdependencies among various parameters of gluten-free sweets composed entirely of quinoa flour, as revealed by principal component analysis. Notably, the parameters of softness, moisture, and color exhibited a highly correlated covariance within the sample. Conversely, the overall acceptance parameter demonstrated a relatively consistent relationship with the protein parameter but remained independent of other parameters. The texture and flavor parameters, however, displayed a non-

collinear association with heightened intensity. Moreover, the specific volume parameter's positioning relative to the textural characteristics of the product suggests a conflicting relationship between these aspects. This indicates that the complete substitution of chickpea flour with quinoa flour (100%) had a detrimental impact on specific volume due to the resulting textural changes. Previous research conducted by Brito et al. (2015) aligned with these findings, showing that increasing the proportion of quinoa flour in sweets negatively affected color, diminished specific volume, and elevated hardness and stiffness in the final product [32].



Figure 5- Analysis of relationships between quantitative and qualitative parameters in gluten-free sweets with 100% substitution of quinoa flour in the principal components.

4-Conclusion

Principal component analysis (PCA) is a factor analysis technique employed to simplify and visualize multidimensional datasets commonly encountered in descriptive analyses. This tool enables the exploration of underlying relationships between quantitative and qualitative product attributes within a vector space. In a recent study, PCA was applied to investigate the relationships between quantitative and qualitative parameters of sweet chickpeas. The study demonstrated the effectiveness of PCA in depicting variations in parameter relationships under varying levels of chickpea flour substitution with quinoa flour. The results highlight the utility of PCA in designing and developing sweet chickpea formulations incorporating quinoa flour. Based on the study findings, a maximum of 50% substitution of chickpea flour with quinoa flour is recommended in chickpea sweets formulations to maintain desired product characteristics and optimize the balance of quantitative and qualitative attributes.

5- References

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مجله علوم و صنايع غذايي ايران





مقاله علم<u>ى پژو</u>هشى

بررسی تأثیر سطوح جایگزینی آرد کینوا بر خصوصیات کمی و کیفی شیرینی بدون گلوتن با استفاده از روش تحلیل مؤلفه اصلی (PCA)

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چکیدہ	اطلاعات مقاله
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و ۱۰۰ درصد) با آرد کینوا بر روابط و وابستگی بین میزان پروتئین، خاکستر، رطوبت، حجم	تاریخ های مقاله :
مخصوص، بافت، تغییرات رنگ و ویژگیهای حسی شیرینی بدون گلوتن با استفاده از روش	تاریخ دریافت: ۱٤۰۲/۸/۲۹
تحلیل مؤلفه اصلی (PCA) انجام شد. نتایج حاصله نشاندهنده وابستگی و رابطه میان	تاریخ پذیرش: ۱٤۰۲/۱۰/۱٦
پارامترهای ارزیابی شده به تغییر در سطح جایگزینی آرد نخودچی با کینوا در فرمولاسیون	
شیرینی بدون گلوتن بود. با جایگزینی ۲۵ درصد روابط میان پارامترها به صورت قابل توجهی	
دچار تغییر شد. با افزایش تغییر سطح جایگزینی از ۲۵ به ۵۰ درصد، با غالب شدن بو و مزه	کلمات کلیدی:
و تغییر در بافت، روابط میان پارامترهای ارزیابی شده در فضای تحلیل مؤلفههای اصلی،	بافت،
دچار بازآرایی نسبت به یکدیگر شدند. با افزایش سطح جایگزینی از ۰۰ به ۷۵ درصد،	بدون گلوتن،
تأثیرگذاری حضور آرد کینوا بر خصوصیات بافتی فراورده تولیدی و روابط آن با سایر	تحلیل مؤلفههای اصلی،
پارامترها، پراکندگی صفات مورد بررسی را نشان داد. این امر بیانگر سطح بحرانی تغییر	کینوا،
روابط به سمت غالب شدن خصوصیات کینوا در فرمولاسیون بود. در سطح جایگزینی ۱۰۰	ویژگیهای حسی.
درصد بر مبنای موقعیت قرارگیری پارامتر حجم مخصوص با پارامترهای مربوط به	DOI:10.22034/FSCT.21.149.128.
خصوصیات بافتی، رابطهای متضاد میان آنها مشاهده شد. براساس نتایج حاصله قابلیت	* مسئول مكاتبات:
کاربرد روش تحلیل مؤلفه اصلی به عنوان ابزاری سودمند در شناسایی زیرلایه روابط میان	Baharehsahraiyan@yahoo.com
پارامترها کمی و کیفی در جهت طراحی و توسعه فرمولاسیونهای غذایی را نشان میدهد و	
در نهایت حداکثر جایگزینی ۰۰ درصد از آرد نخودچی با آرد کینوا در فرمولاسیون شیرینی	
نخودچي توصيه مي گردد.	