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# EFFECT OF CHIA (SALVIA HISPANICA L) ADDITION ON THE QUALITY OF GLUTEN-FREE BREAD

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#### **ABSTRACT**

The objective of this study was to analyze the influence of chia seeds and flour on the organoleptic quality of gluten-free breads. Rice breads were elaborated with the addition of 15 g of chia flour or seed, either dry or pre-hydrated, per 100 g of rice flour. The influence of the addition of chia on flour pasting properties and dough proofing behavior was analyzed. The specific volume, weight, texture, color and acceptability of the breads were studied. The addition of chia reduced specific volume and increased the hardness of breads, and the effect was more evident with the flour than with seed. Chia addition minimized weight loss during baking in all cases. The addition of chia flour produced a darker crust and crumb. There were no significant differences between the different breads in the global acceptability, but chia seed breads presented better values in terms of texture than control.

#### PRACTICAL APPLICATIONS

This study shows that the use of chia seed and flours in rice bread making allows results in breads with good acceptability. However, the correct selection of seed or flours and previous hydration or not are essential to achieve appropriate results. Thereby, breads with chia seeds showed a better aspect valued by consumers, a higher specific volume and a lower firmness than breads made with chia flour, without a quality improvement in the case of a previous prehydration.

#### INTRODUCTION

Celiac disease (CD), also known as celiac sprue or glutensensitive enteropathy, is an inflammatory disease of the upper small intestine triggered by the ingestion of gluten, a protein present in wheat, rye, barley and possibly oats. It is one of the most common food intolerances, affecting 1% of the population worldwide (Catassi and Yachha 2009). The characteristic feature of CD is flattening of the intestinal villi, which reduces their surface area and results in nutrient malabsorption. Ever more people follow a glutenfree diet, both because there has been an increase in the number of individuals diagnosed with the disease and because it has been demonstrated that gluten intake causes gastrointestinal symptoms in some individuals without CD (Biesiekierski *et al.* 2011) – or because of false beliefs. This has led to a large increase in the market for gluten-free

bakery products in recent years (Cureton and Fasano 2009).

It has been widely reported that a gluten-free diet can be unbalanced, with an excess of saturated fats and a lack of fiber, iron and calcium (Mariani *et al.* 1998; Thompson *et al.* 2005; Hopman *et al.* 2006; Kinsey *et al.* 2008; Wild *et al.* 2010). One of the reasons for these shortcomings is that gluten-free cereal-based products tend to be made with refined flour or starches, and they are not usually enriched or fortified. The design of a gluten-free diet cannot therefore be limited simply to the avoidance of gluten consumption, but must also ensure an adequate intake of essential nutrients.

A number of studies have investigated the inclusion of different seeds into gluten-free breads in order to increase their nutritional value. Korus *et al.* (2012) added defatted strawberry and blackcurrant seeds, Bernardi *et al.* (2010)

used *Prosopis ruscifolia* (vinal) seeds, and Alvarez-Jubete *et al.* (2009, 2010) replaced cereal flours and starches with pseudocereal flours. The inclusion of different fibers into the formulations has also been studied (Sabanis *et al.* 2009; Hager *et al.* 2011).

Chia seeds have a high fat content (over 30 g oil/100 g) with a low proportion of saturated fatty acids, and a high level of linoleic acid (60.5-67.8 g/100 g fat) (Coates and Ayerza 1996; Ixtaina et al. 2011; Martinez et al. 2012). These seeds also contain high levels of protein (19.0–26.5 g/100 g), fiber (37.0-59.8 g/100 g), mostly insoluble, and antioxidant substances (Weber et al. 1991; Reves-Caudillo et al. 2008; Capitani et al. 2012). Because of its nutritional composition, particularly the omega-3 fatty acid, fiber and antioxidant content, chia is an ideal seed for the enrichment of cereal products, and there has been an increase in the number of commercial products that incorporate chia in their formulation in recent years. Ayerza and Coates (2005) demonstrated that chia diets dramatically decreased triacylglycerol levels and increased high-density-lipoprotein cholesterol and omega-3 fatty acid levels in rat serum. The beneficial effect of chia on lipid and glucose homeostasis has also been demonstrated in an experimental model of dyslipidaemia and insulin resistance (Chicco et al. 2009). When hydrated, the seed of chia releases mucilage, which is located in the seed coat (Muñoz et al. 2012). This mucilage or gum has a molecular weight of 0.8-2 × 10<sup>6</sup> Da (Lin et al. 1994) and a powerful thickening effect, and it has therefore been used to replace eggs and oil in cake making (Borneo et al. 2010).

The objective of this study was to investigate the influence of the incorporation of chia seed on the quality of gluten-free breads made with rice flour. The whole or ground seed was incorporated directly or after prior hydration in order to extract the mucilage from the seed. The volume and weight of the breads, the texture and the crumb and crust color have been assessed. We also performed rheofermentographic analysis to evaluated dough behavior during fermentation and carried out a test of sensory acceptability with potential consumers.

#### **MATERIALS AND METHODS**

#### **Materials**

Rice flour was supplied by Harinera Castellana S.A. (Medina del Campo, Valladolid, Spain). The flour was sieved and the 132–200  $\mu$ m fraction (8.02 g of protein/100 g) was used. The color of the rice flour was L=93.49, a=-0.37 and b=6.92. Chia seed (3.78% moisture, 23.90% protein and 32.86% fat) was provided by Trades (Barcelona, Spain). Coarse flour was used by their better behavior in rice bread making (de la Hera *et al.* 2013). Chia seed was ground, as required, with a super junior "s" grinder (Moulinex,

Selongey, France). The color of the chia flour was L = 46.25, a = 3.58 and b = 11.92. Salt, sugar and sunflower oil were purchased from the local market. Dry yeast (Saf-Instant, Lesaffre, Lille, France) and hydroxypropyl methylcellulose (HPMC) (Methocel K4M, Dow Chemical, Midland, MI) were used.

Fat determination in chia seeds was performed in accordance with American Association for Clinical Chemistry (AACC) method 30-25, and the moisture and protein content of chia seed and the rice flour in accordance with methods 44-15 and 46-11 (AACC International 2012), respectively. The color of the flours was measured using a Minolta spectrophotometer CN-508i (Minolta, Co. Ltd, Tokyo, Japan) under the D65 standard illuminant and with the 2° standard observer. All measurements were made in duplicate.

#### **Methods**

**Bread Making.** A straight-dough process was employed, using a Kitchen-Aid Professional mixer (KPM5, KitchenAid, St. Joseph, MI) with a dough hook (K45DH). Three different formulas were tested. The following ingredients (as % on rice flour basis) were used in all formulas: water (100%), sunflower oil (6%), sucrose (5%), salt (1.8%), instant yeast (3%) and HPMC (2%). In all cases, the instant yeast was first rehydrated in 200 mL of the total volume of water for the dough. Seven hundred grams of flour was used in each batch. Chia seed and chia flour was added at a ratio of 15 g per 100 g of rice flour. The percentage of added chia was determined in basis on previous studies, since higher percentages of chia gave rise to breads with a muddy texture. When the chia seed or chia flour was prehydrated, this was performed with the total amount of water in the formula (except for the 200 mL used in yeast prehydration) for a period of 30 min at 20-22C. The dough was kneaded for a period of 8 min at speed 2 (Gomez et al. 2013). Each dough was prepared in duplicate. The doughs were molded into aluminum pans of 232 × 108 × 43.5 mm: 400 g into each pan. Pans were placed into a proofing chamber at 30C and 90% relative humidity for 90 min. After proofing, the breads were baked in an electric oven for 40 min at 190C. After baking, breads were demolded, cooled for 60 min at room temperature and packed into sealed polyethylene bags to prevent dehydration.

**Dough Measurements.** The effect of chia addition on the viscosity and pasting properties of flour was analyzed using the standard method with a Rapid Visco™ Analyser (RVA) (Newport Scientific Pty Ltd., Warriewood, Australia) (AACC method 76-21, 2012), using the Rapid Visco Analyser RVA-4 (Newport Scientific Pty. Ltd.) controlled by *Thermocline for Windows* software (Newport Scientific Pty. Ltd.).

The effect of chia addition on dough proofing was determined using a rheofermentometer (Chopin, Villeneuve-la-Garenne, France), obtaining information on dough development and gas production during fermentation (Czuchajowska and Pomeranz 1993). In contrast to the traditional method, the weight of dough was reduced to 200 g and the weights were removed from the piston due to the weakness of this kind of dough compared with one prepared with wheat flour. The dough used in the analysis was elaborated using the same method as described earlier and the proofing temperature was set at 30C, as in the bread making. The proofing conditions were therefore as similar as possible to those used for the doughs that were baked.

**Bread Characteristics.** Bread quality was evaluated 24 h after baking. Bread volume was determined using a laser sensor with the BVM-L 370 volume analyser (TexVol Instruments, Viken, Sweden). The bread specific volume was calculated as the ratio between the volume of the bread and its weight. Weight loss was measured as the difference between the weight of the dough as molded and the weight of the bread after baking. Measurements were performed in duplicate.

Crumb texture was determined using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) running the "Texture Expert" software. A 25-mm diameter cylindrical aluminum probe was used in a "texture profile analysis" (TPA) double compression test to penetrate to 50% of the depth at a speed of 2 mm/s and with a 30-s delay between the first and second compressions. Firmness (N), cohesiveness and springiness were calculated from the TPA graph. Measurements were made on two central slices (20 mm thickness) from two breads from each dough.

Color was measured using a Minolta spectrophotometer CN-508i (Minolta, Co. Ltd). Results were expressed in the CIE  $L^*$   $a^*$   $b^*$  color space and were obtained under the D65 standard illuminant with the  $2^\circ$  standard observer. Color determinations were made  $5\times 5$  times on each piece of bread: crumb and crust color was checked at five different points on each piece of bread and each point was measured five times.

Consumer Testing. Hedonic sensory evaluation of the breads was conducted with 54 bread-usual-consumer volunteers (24 men and 30 women between 18 and 55 years of age). The volunteers were recruited from the staff and students of the Agricultural Engineering College of the University of Valladolid, Palencia, Spain, and came from different socioeconomic backgrounds. Consumer tests were carried out in individual booths in the Sensory Science Laboratory of the Agricultural Engineering College. This method (rather than mobile laboratory tests, central location tests, or home-use tests) was selected to afford better control of the experimental conditions and of sample preparation.

Volunteers were asked to evaluate the gluten-free breads according to their appearance, smell, taste, consistency, texture and overall acceptability on a 9-point hedonic scale. The scale of values ranged from 1, "dislike extremely", to 9, "like extremely." Samples were analyzed one day after baking and were presented as 2-cm slices on white plastic dishes coded with a random three-digit number and served in random order. All breads were analyzed in the same session. Water was available for rinsing.

#### **Statistical Analysis**

Data were processed using analysis of variance (ANOVA). Control bread (without chia addition) and breads with the incorporation of a 15% of chia, both as flour and seed, with and without prehydration  $(1+2\times2)$ , were made. Elaborations were made in duplicate. Fisher's least significant differences (LSD) test was used to describe means with 95% confidence. Statgraphics Plus V5.1 software (Statsoft Inc., Tulsa, OK, USA) was used for the statistical analysis.

#### **RESULTS AND DISCUSSION**

#### **Dough Characteristics**

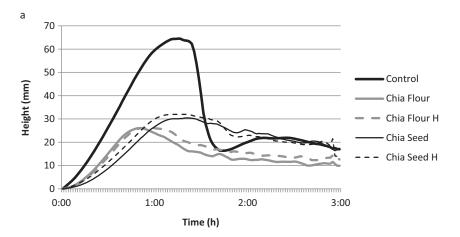
Table 1 shows the effect of the addition of chia on the pasting properties of the flour. The addition of 15% chia seed increased the pasting viscosity, peak viscosity and final viscosity, whereas the inclusion of chia flour did not alter pasting or peak viscosity but reduced final viscosity. In the case of chia seed, which has been shown to release mucilage, the increase in viscosity may be due to the interaction of this substance with the starch, matching the effect of hydrocolloids on pasting properties (BeMiller 2011). However in the case of ground chia, the lipid-starch interaction, which depends on the composition of fatty acids, must be added to the interaction between the hydrocolloid and starch (Nierle and Elbaya 1990). Both the flour and the seed decreased the pasting temperature and the peak time, but the decrease in pasting temperature was smaller in the case of seed. Seed without prehydration did not show significant differences from prehydrated seeds, except for pasting temperature, which was higher. This result would appear to indicate that modification of the pasting temperature is due mostly to the effect of chia mucilage, in agreement with the statement by BeMiller (2011) on the effect of hydrocolloids on the temperature of the initial rapid increase in viscosity. The inclusion of chia flour had a greater effect than the seed on breakdown and setback, decreasing both parameters. This effect cannot be attributed to the presence of lipids, as lipids tend to increase the setback of rice starch (Liang et al. 2002). However, it may be attributed to the presence of hydrocolloids, although the literature on the subject is

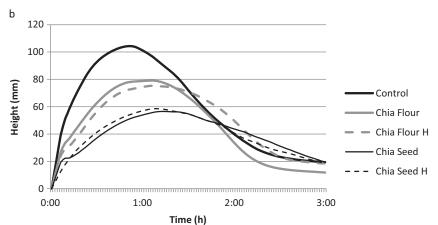
TABLE 1. EFFECT OF CHIA ADDITION ON RVA PARAMETERS

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	Pasting viscosity (cp)	Pasting viscosity (cp) Pasting temperature (C)	Peak viscosity (cp)	Peak time (min)	Peak temperature (C)	Breakdown (cp)	Trough (cp)	Setback (cp)	Final viscosity (cp)
Control	ol 25.0a	74.7c	5088a	5.70b	95.05a	2132c	2956a	2867d	5823b
Seed	133.5bc	73.0c	5435b	5.27a	94.95a	1939bc	3497d	2547c	6044c
Seed H	204.0c	69.1b	5468b	5.27a	94.93a	2040c	3429cd	2550c	5979c
Flour	115.5abc	66.2a	4805a	5.37a	94.95a	1529a	3277bc	2101a	5378a
Flour H	66.0ab	65.3a	4871a	5.37a	94.98a	1740ab	3131b	2286b	5417a

Different letters in the same column indicate significant differences (95% level) (Fisher's test) H, prehydration; RVA, Rapid Visco Analyser. contradictory, and while Song et al. (2006) stated that small doses of hydrocolloids do not modify setback, Arocas et al. (2009) found an increase in setback with the addition of hydrocolloids. In the case of chia seed, there was no significant difference in breakdown compared with the control, but there was a decrease in setback, as found with the flour. The inclusion of chia flour also increased the trough, though to a lesser extent than the inclusion of seed. In general no significant differences were observed between the inclusion of flour and seeds with and without prehydration, with the exception of pasting temperature in the case of seed and setback in the case of flour.

Figure 1a shows the development of the dough during fermentation. The control curve showed a greater and higher rise, but fell abruptly at around 90 min. Therefore, doughs with short fermentation times made with chia flour or seed show less development during fermentation but, under these conditions, the height of the dough with seed was equivalent when long fermentation times were used. Therefore tolerance in this type of dough is greater than the control. Doughs prepared with seed presented a greater maximum height, although this was reached somewhat later than in the doughs prepared with flour; this indicates an earlier breakage of the dough structure and loss of the gas produced in the case of doughs prepared with flour. With regard to volume fall with excess fermentation time, it can be seen that the drop was much less pronounced with the doughs with chia flour than with the control dough, and slightly less pronounced with the doughs with chia seed. Prehydration did not produce any major differences with either flour or seeds. Differences in the increase in volume of the dough and, therefore, in gas retention may be due to changes in the internal structure of the dough and the way in which the hydrocolloid (HPMC) interacts with the particles that form the dough; previous studies have found that a proportion of these particles remain in the final dough in their original size and shape (de la Hera et al. 2012) and this may affect internal cohesion in the structure of the dough. These differences, in the case of flour, may also be related to the release of large amounts of fiber and oil. Oil interacts with the water and hydrocolloid in the solution that is attached to the particles, whereas the fiber tends to swell (extensively) and can weaken the structure of the dough as suggested by Moore et al. (2004). Gas production (Fig. 1b) was found to be faster in the control dough, but also decreased earlier, probably due to the lack of fermentable sugars. This faster production can also contribute to an earlier and sudden fall of the dough due to the generation of a more airy and weaker structure. The doughs that incorporated chia seeds presented more gradual gas production, and the doughs with chia flour showed an intermediate behavior. There were no major differences according to whether or not the seeds or flour were prehydrated, which





**FIG 1.** DOUGH DEVELOPMENT CURVE: OBTAINED WITH THE RHEOFERMENTOMETER (a) Dough development. (b) Gas production.

may indicate that in this type of dough, with a high moisture content and less competition for water, there is a release of mucilage within the dough similar to that achieved by prehydration.

# **Bread Properties**

The addition of chia flour or seed tended to produce a reduction in the specific volume of breads and less weight loss during baking (Table 2). The results of studies of the addition of flaxseed flour (which has a similar composition

to chia) to wheat breads in percentages similar to those used in our study have been contradictory; while some authors have detected no reduction in specific volume (though they do report a modification of dough rheology) (Koca and Anil 2007), others claim that the incorporation of these flours reduces bread volume (Conforti and Davis 2006). However, we cannot compare the results of those studies with our results because of the gluten network functionality in that type of bread and because of the option to modify the hydration of the dough according to the farinographic values. The decrease in the specific volume observed in our

**TABLE 2.** EFFECT OF CHIA ADDITION ON BREAD CHARACTERISTICS

	Specific volume (mL/g)	Weight loss (g/100 g)	Firmness (N)	Cohesiveness	Springiness
Control	6.02c	22.91c	0.67a	0.39a	0.74a
Seed	3.00b	15.70b	6.41b	0.35a	0.72a
Seed H	3.01b	13.80ab	8.42b	0.44a	0.81a
Flour	2.28a	13.68ab	22.49c	0.38a	0.80a
Flour H	2.27a	13.37a	21.52c	0.38a	0.80a
Seed H Flour	3.01b 2.28a	13.80ab 13.68ab	8.42b 22.49c	0.44a 0.38a	0.81a 0.80a

Different letters in the same column indicate significant differences (95% level) (Fisher's test). H, prehydration.

study was more evident with chia flour than with the seed. This was due in part to the lower development of the dough during fermentation, as can be seen in the rheofermentometer curves, and in part to less expansion during baking due to a reduction in the pasting temperature, as the dough expands in the oven until the bread structure becomes rigid due to starch gelatinization and other factors (Stauffer 1990). This smaller volume, in the case of the addition of chia flour, could be due to the lower percentage of water in the formulation, because larger amounts of water in the formulation produce breads with larger volumes (McCarthy et al. 2005). In the case of chia seed, which interacts less with the dough, this effect is minimized. Breads without chia present a greater loss of water in the baking process. This effect can be partially attributed to their greater volume and therefore a larger surface area for exchange with the exterior. Among chia breads, the greatest loss of moisture was observed in breads with chia seed without prehydration. This would appear to demonstrate that the cause of greater water retention is chia seed mucilage, which is most effectively released from the flours (with a greater available surface area for exchange) and from prehydrated seed (with more time for mucilage release). The direct consequence of this lower loss of moisture in baking is a higher final humidity of bread after the incorporation of the different forms of chia.

In general, breads with added chia showed greater firmness (Table 2). A higher firmness is also observed in breads with the addition of chia flour, against those with chia seed. It can be stated that the effect on firmness is heavily influenced by the differences in specific volume, so that the higher the specific volume, the lower the firmness of the loaves. This correlation has been observed previously both in bread with gluten (Gomez et al. 2008) and in batters, more similar to gluten-free breads (Gomez et al. 2010). There are no differences between cohesiveness, and springiness bread color was most affected by the addition of chia flour in either of its forms; these flours led to a fall in the  $L^*$ (luminosity) parameter of the crumb and an increase in the values of  $a^*$  and  $b^*$  (Table 3). The internal part of the dough cannot reach a temperature higher than the temperature of evaporation of water, and Maillard reactions and sugars

caramelization therefore do not occur; the color of the bread crumb thus depends mainly on the color of the raw materials, as has been observed in other studies (Gomez et al. 2011). In our case, the differences were due to the gray color of the chia flour, which "stain" bread made with rice flour. On the other hand, chia seeds are distributed throughout the dough and do not produce an overall color change. In the crust, there was a decrease in brightness after adding chia flour, and also a decrease in the values of  $a^*$  and  $b^*$ . In the crust, the temperature reached allows Maillard reactions and sugar caramelization to occur and therefore there may be differences in color apart from those due to the color of the raw materials themselves, and this will depend on the effect of the ingredients on the Maillard reactions, because of their different content of amino acids and reducing sugar (Purlis 2010). Chia flour has higher protein content than rice flour and a different amino acid composition. When chia seed is added, those components are protected and do not interact with the components of the rice flour and therefore do not intervene in the coloration of the crust.

## **Sensory Evaluation**

Table 4 shows the organoleptic assessment of breads. Sensory evaluations are always important, but particularly in the case of gluten-free breads because of the poor correlation observed between sensory perception and instrumental measurements, most evident in parameters such as taste and smell, but also affecting appearance or texture (Matos and Rosell 2012). The table shows that there are no differences between the breads in terms of overall acceptability, and all of them slightly exceed a score of 5 (neither like nor dislike). It must be taken into account that the consumer tasting participants were not celiacs and were accustomed to eating bread made with wheat flour. Changes both in the flavor and in the texture of the gluten-free breads negatively influenced overall evaluation, but it was noticeable that the addition of a high percentage of chia (15%) did not change the overall evaluation. Nor were differences detected in the smell or flavor persistence of the bread. However, the texture of breads made with chia seed was preferred to that

Crumb Crust L\* b\* L\* Dough hydration a\* a\* h\* Control 73.96b -0.12a 2.84a 63.02b 8.68b 25.16b 68.71b 0.01a 6.88ab Seed 4.67b 65.67c 23.35b Seed H 71.92b 0.02a 4.88b 69.46d 5.69a 22.16b 61.21a 2.36b 9 1c 59.48a 5.73a 17.77a Flour H 60 29a 5.65a 17.54a

**TABLE 3.** EFFECT OF CHIA ADDITION ON BREAD COLOR

Different letters in the same column indicate significant differences (95% level) (Fisher's test). H, prehydration.

**TABLE 4.** EFFECT OF 15% CHIA ADDITION ON ORGANOLEPTIC ACCEPTABILITY OF BREADS (100% HYDRATION)

	Overall acceptability	Smell	Taste	Persistency	Texture	Appearance
Control	5.32a	5.85a	5.36ab	5.28a	4.74a	5.98ab
Seed	5.76a	5.74a	5.65b	5.22a	5.56b	6.20b
Seed H	5.59a	5.87a	5.43ab	5.30a	5.50b	6.20b
Flour	5.39a	6.17a	5.11a	5.11a	5.24ab	5.54a
Flour H	5.31a	5.87a	5.24ab	5.28a	5.31ab	5.56a

Different letters in the same column indicate significant differences (95% level) (Fisher's test). Scale values ranged from 9 "like extremely" to 1 "dislike extremely". H, prehydration.

of the control bread. On comparison of the inclusion of seeds or flour, the appearance of the bread with seeds scored higher than breads with flour, possibly due to the grayish color of the crumb, which is unusual in bakery products, whereas the seed produced a mottled appearance, which is more common in conventional bakery products. With regard to the flavor of the breads, the addition of seeds without prehydration obtained a higher valuation than flour without prehydration, but ratings were equal for the prehydrated forms. In general, chewing the seeds produces a release of flavors in the mouth which does not occur when the milled product is consumed, and this can cause differences in the evaluation. However, there were large differences between consumers, as 26% clearly preferred breads made with chia seed over those made with flour, whereas 11% preferred breads made with chia flour (data not shown). The other parameters studied showed no differences between the addition of seeds or flour, with or without prehydration. Although, on comparison with the control bread, the chia seed bread had a lower volume and greater firmness, which are aspects commonly assessed in this type of study and usually determine the final quality of a product, these parameters did not influence acceptability of the products; thus, there must be more important factors that significantly affect the acceptability of bread with chia, such as flavor or the mouth sensations.

#### **CONCLUSIONS**

The incorporation of chia may be an interesting way to improve the nutritional characteristics of gluten-free breads, as the incorporation of 15% chia does not reduce sensory acceptability of these products. The inclusion of chia reduced the specific volume and increased the firmness of breads, but these changes were diminished when chia was incorporated as seed, against breads with chia flour. The flour inclusion also increased the differences among the crumb and crust color, producing darker colors, with a less valued aspect by consumers against breads with chia seeds.

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