

Gluten-Free Flat Bread and Biscuits Production by Cassava, Extruded Soy Protein and Pumpkin Powder

Mona M. A. Aly, Hinar A. Seleem*

Department of Crops Technology Research, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt
Email: hanhona853@hotmail.com

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Abstract

In recent years, there has been an increase in demand of gluten free products that are suitable for people with celiac disease. The present study was carried out to produce gluten free flat bread and biscuits with good quality. The ingredients under this study were cassava flour, rice flour, extruded soy protein (ESP) and pumpkin powder. Four levels of ESP were used for production of flat bread and biscuits: 2.5%, 5%, 7.5% and 10% for flat bread and 5%, 10%, 15% and 20% levels for biscuits. Results of flat bread samples showed that protein, fat, ash and fiber contents increased in all samples as increasing the level of ESP. Flat bread at level 10% ESP had the highest value of β -carotene. Alkaline water retention capacity (AWRC) at zero time and 24 h of flat bread storage had high values for levels 2.5% and 5% ESP. Water holding capacity (WHC) increased insignificantly by increasing the level of ESP. Color measurements revealed that the lightness decreased and the redness increased with increasing the level of ESP. Sensory evaluation of flat bread revealed that 2.5% followed by 5% ESP level had high score of overall acceptability. Physical properties of biscuits indicated that as the level of ESP increased the diameter, thickness, volume and specific volume decreased. Biscuits sample with 20% ESP had the highest values of protein, fat, ash and fiber but the lowest in total carbohydrates. Also β -carotene and vitamin A content increased in biscuit samples. Caloric values of biscuits in all treated samples were lower than control. Lightness decreased while redness increased with increasing the level of ESP. Data of texture profile analysis (TPA) showed that hardness and adhesiveness (g) increased as ESP level increased. Sensory evaluation of biscuits showed that addition of ESP at 20% level decreased significantly texture score from 9.51 to 6.61 ($P < 0.05$) but insignificantly affected the other sensory scores.

Keywords

Celiac Disease, Rice Flour, Cassava Flour, Extruded Soy Protein, Pumpkin Powder, β -Carotene,

*Corresponding author.

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Color, Texture Profile Analysis, Flat Bread, Biscuits

1. Introduction

Celiac disease (CD) is an autoimmune and chronic disorder in which the mucous membrane of the small intestine is damaged in gluten-intolerant individuals. CD is caused by not only a reaction to gliadin in wheat prolamin but also high molecular glutenin and subunits of gluten protein consequents in damage and inflammation to the small intestine and causes malnutrition [1]. One person out of two hundred has been diagnosed with this disease and some studies have stated that the dominant of this disease is 1 in 100 worldwide [2]. This chronic disease is recognized as long-life disease and the only solution is adherence stickiness to gluten-free products. But this is not easy as many foodstuffs contain gluten [3]. Middle Eastern countries and many of families in the world now consume the flat bread type. In general, flat bread is a simple formula produced by a few ingredients. It is consumed either by incorporating foods such as meat or vegetables in the dough or as final products in which food can be placed in the pocket of the two layer flat bread. A correct balance of viscoelastic properties is considered to be an important during flat bread making [4].

Rice is the main staple food for many countries, providing 20% of the food energy supply in the world. It is known as queen among cereals after wheat. Rice is characterized by low prolamin, hypoallergenic activity, insipid taste, low sodium and high digestible carbohydrate contents, which is suitable to be incorporated into celiac diets [5].

Rice flour, corn and cassava starches were used to obtain non-gluten bread. The type of starches (granule size, amylose/amylopectin content, chemical, and physical modification) could influence the batter consistency and the gelatinization-retrogradation rate; these parameters strongly related to bread quality [6].

Cassava flour can be used for many people suffering from celiac disease, a chronic enteropathy characterized by an inadequate immune response to ingested gluten from wheat, rye, barley, and triticale [7]. Reduction in the consumption or outright elimination of gluten-free foods would be desirable [8]. Cassava flour (CF) is one of the major products from cassava roots treated in the world food market [9]. Cassava flour has also continued to find wider applications in foods, feed and chemical industries [10].

In particular, proteins isolated from legumes and dairy sources are most often added to gluten-free products [11].

Hydrocolloids are substances that are used as additives for the purpose of reproducing similar viscoelastic properties to the gluten. They are water-soluble polysaccharides, with varied chemical structures that confer certain properties that make it of a suitable functional application [12]. Hydrocolloids such as pectin, guar gum and xanthan gum are added to naturally gluten-free flours to mimic the viscoelastic properties of gluten and to improve structure, sensory attributes and shelf-life of these products [11].

Pumpkins are extensively grown in tropical and subtropical countries. Traditionally it is consumed as freshly boiled, steamed or processed food items such as soup or curry. Pumpkin is high in β -carotene, which gives its yellow or orange color [13]. Consumption of foods containing carotene helps in prevention of eye disorders, cancer and skin diseases [14]. Incorporation of β -carotene rich foods in diets is the best measure to improve vitamin A nutrition of individuals to overcome the problems and diseases caused by vitamin A deficiency [15].

The development of good quality gluten free bread is a serious task. Therefore, many researchers have investigated the substitution of gluten by ingredients able to mimic its functional properties [16].

Bread is a staple diet that is consumed daily and its quality and sensory attributes are highly considered by consumers. But the quality of the gluten-free bread might be different than conventional heat bread due to lack of gluten [3].

Bread staling involves crumb firming and has been attributed to several factors including recrystallization of amylopectin, water redistribution, and the state of amorphous phase. In wheat bread, the gluten network slows down the movement of water from the bread crumb to crust, and thus the lack of this structure in gluten-free bread should enhance the water movement resulting in bread that is more prone to stale [17].

Gluten-free biscuits are typically round cakes of bread that are leavened with baking powder, baking soda or sometimes yeast. It may also refer to cookies or crackers. They are mostly sweet and in history they were used by travelers as they were long-lasting foods and easy to carry [18].

The diet of celiac patients must be completely free of any gluten, so all the products from wheat, rye, barley and oat must be replaced with corn, rice, millet equivalents and various types of starch (corn, rice and potato) or appropriate mixtures [11].

This investigation aimed to use cassava flour and extruded soy protein (ESP) in preparing gluten-free flat bread and biscuits with high nutritional value for people suffering from celiac disease.

2. Materials and Methods

2.1. Materials

Broken rice supplied by Rice Milling Company, Dakahlia, Egypt. Cassava Flour was obtained from Royal House Market, Heliopolis, Cairo, Egypt. Extruded soy protein (ESP) was obtained from AWA for food additives company Alex., Egypt. Pumpkin vegetable, salt, sugar powder, butter, eggs and baking powder are purchased from local markets, Cairo, Egypt. Xanthan was obtained from Doves farm foods Co. UK.

Chemicals

All chemicals used were purchased from Algomhorya Company, Giza, Egypt.

2.2. Preparation of Raw Materials

2.2.1. Rice Flour

The broken rice was directly ground into flour using the mixer grinder and sieving through 40 mesh sieve and placed on a cooling rack in refrigerator [19].

2.2.2. Pumpkin Powder

The peeled pumpkin was converted into 10mm size cubes then cut into slices (2 mm) and subjected for pre-treatments such as blanching (94°C for 2 min) then dried for preparation of powder using vacuum dryer (Vacuum Oven ADP-31 Made In Japan). Vacuum drying of pumpkin slices was carried out at 80°C and 700 mm Hg vacuum. The dried pumpkin was milling and sieving through an 40-mesh and placed on a cooling rack in refrigerator [20].

2.2.3. Preparation of Products

1) Flat bread

The flat bread blends shown in **Table 1**. Formulas were treated, hydrothermally (as a preliminary study) to reach the quantity of warm water (60, 65, 70, 75 and 80 ml). For preparation flat bread dough, warm water

Table 1. Formulation and added ingredients for flat bread and biscuits.

Ingredients (g)	Flat bred					Biscuits				
	1	2	3	4	5	1	2	3	4	5
Rice flour	50	50	50	50	50	50	50	50	50	50
Cassava flour	50	50	50	50	50	50	50	50	50	50
ESP*	0.0	2.5	5.0	7.5	10	0.0	5	10	15	20
Pumpkin powder	0.0	7	7	7	7	0.0	7	7	7	7
Xanthan	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	1	1	1	1	1	-	-	-	-	-
Sugar	-	-	-	-	-	85	85	85	85	85
Whole egg	-	-	-	-	-	52	52	52	52	52
Baking powder	-	-	-	-	-	5	5	5	5	5
Butter	2	2	2	2	2	80	80	80	80	80
Warm water (ml)	60	65	70	75	80	-	-	-	-	-

*ESP: Extruded soy protein.

added to formulated flour and gelatinized for 3 min on Steam cooker. The dough of each formula divided into 15 g pieces. Then, every piece of dough shaped, into roller shape (2 mm thick and 15 mm diameter) and baked in electric oven at 250°C for 3 min. Then air cooled, packed in polyethylene bags.

2) Biscuits

The biscuit blends shown in **Table 1**. It prepared according to the methods described by Oyewole *et al.* [21]. Butter and sugar were mixed in (a Kenwood mixer) at a medium speed until a light and fluffy cream was formed, added egg and continue the mixing. Cassava flour, rice flour, (ESP), pumpkin powder and xanthan were slowly added to the mixture then rolled on a flat rolling board. Circular biscuits were cut, placed on greased baking trays and baked in an electric oven (Kumatel, Turkey) at 160°C for 15 min.

2.3. Analysis

2.3.1. Chemical Composition

Proximate analysis including moisture, protein, fat, ash and crude fiber were carried out according to the methods of AOAC [22]. Carbohydrates content was calculated by difference.

2.3.2. β -Carotene Content

β -carotene was determined by using water-saturated *n*-butanol (WSB) according to the method outlined by Santra *et al.* [23] A calibration curve was made from known quantities of β -carotene. β -carotene content was expressed as $\mu\text{g}/100$ on dry weight basis. Vitamin A value calculation was performed based on vitamin A activity of the β -carotenes according to the conversion factor provided by the Food and Nutrition Board, Institute Of Medicine [24]. Vitamin A value was expressed in retinol activity equivalents (RAE), which represents vitamin A activity as retinol. Where, 12 μg of β -carotene from foods are required to provide the body with 1 μg of retinol, giving dietary β -carotene an RAE ratio of 12:1.

2.3.3. Alkaline Water Retention Capacity of Flat Bread during Storage

Alkaline water retention capacity (AWRC) values of flat bread were measured at 0, 24, 48 and 72 h of bread storage at -20°C according to Yamazaki [25] and modified by Kitterman and Rubenthaler [26].

2.3.4. Water Holding Capacity (WHC) of Flat Bread

Water holding capacity (WHC) was performed according to the method of Beuchat [27].

2.3.5. Total Calories of Flat Bread and Biscuits

Total calories of flat bread and biscuits were calculated from the following equation as reported by James [28]. Energy value = 4 (g protein + g carbohydrates) + 9 (g fat).

2.3.6. Physical Properties of Biscuits

The diameter and thickness of biscuits were measured with a venire caliper. Spread ratio was calculated from the ratio of diameter to thickness as described by Gains [29] method. The average of weight biscuit (5 piece) was measured in (g). Volume (cm^3) was measured by displacement of rapeseeds and specific volume was determined by dividing volume (cm^3)/weight (g). Density was calculated by dividing weight (g) out volume (cm^3) and expressed as (g/cm^3) [30]. The specific volume of biscuits was determined according to the method described in AACC [31].

2.3.7. Color Measurements of Flat Bread and Biscuits

External color of the products was measured according to the method outlined by McGurie [32] using a handheld Chromameter (model CR-400, Konica Minolta, Japan).

2.3.8. Texture Profile Analysis (TPA) of Biscuits

Hardness and adhesiveness of biscuits were measured by using Brookfield Engineering Lab. Inc., Middleboro, MA 02346-1031 USA [31].

2.3.9. Organoleptic Evaluation of Products

1) Flat bread

Flat bread samples were organoleptically evaluated for its sensory characteristics. Half slice of each bread sample was served for ten panelists on white, odor and disposable plates. Samples were scored for, taste, chewing ability, texture, aroma, color and overall acceptability using a score from 1 to 10. The evaluation was carried out according to the method of Land and Shepherd [33].

2) Biscuits

Biscuit samples were organoleptically evaluated by ten panelists for its sensory characteristics: appearance, color, odor, texture, taste and overall acceptability as the method described by Larmond [34]. The maximum score of each attribute was (10) degrees.

2.4. Statistical Analysis

The obtained data from chemical, physical and sensory evaluation were exposed to analysis of variance (ANOVA). Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means.

3. Results and Discussion

3.1. Chemical Composition of Raw Materials

The proximate composition of raw materials used for the preparation of bakery products (flat bread and biscuits) is shown in **Table 2**. The obtained data revealed that the highest content of protein found in extruded soy protein (ESP) 56.82%. While, the lowest value was found in cassava flour (1.05%). Fat content ranged from 0.62 up to 4.00% for cassava flour and (ESP), respectively. The values were obtained agreed with those reported by Tharise *et al.* [35] and Ogunjobi and Ognwolu [9]. Pumpkin powder had the highest content of ash 5.83% followed by ESP (4.78%). These results agreed with Pongjanta *et al.* [36]. Fiber content was (1.94%, 1.09%, 2.47% and 2.98%) for rice flour, cassava flour, (ESP) and pumpkin powder, respectively. Cassava flour, rice flour and pumpkin powder had high carbohydrates content 95.83, 89.38 and 84.30%, respectively. Whiles, ESP had the lowest value 31.92%. These results are in agreement with Tharise *et al.* [35].

3.2. Flat Bread

3.2.1. Chemical Analysis of Flat Bread

The chemical composition of flat bread are given in **Table 3**. Data showed that protein content increased as increase ESP level. The highest value was 9.15% for sample No. 5 (10% ESP). On the other hand, fat, ash and fiber contents were increased significantly in all samples. This may be due to the addition of ESP. These results agreed with Hegazy *et al.* [37] who showed that the chemical composition of gluten free bread samples evident increase in protein, fat and ash contents in all gluten free bread samples, probably due to addition of soy flours. Carbohydrate content decreased significantly in all same samples compared with control (sample No. 1). This may be attributed to the increment in protein content.

Carotenoids have been extensively studied due to their important biological functions for humans and also as natural pigments. Relations between carotenoid and vitamin A were found, and some of them have provitamin A activity (α -carotene, β -carotene, γ -carotene, β -zeacarotene and others), which could be transformed in vitamin A inside the animal organism [38].

Data in **Table 3** represents β -carotene $\mu\text{g}/100\text{g}$ flat bread. It could be noticed that β -carotene content ranged

Table 2. Chemical composition of raw materials (g/100g) on dry weight basis.

Parameters (%)	Rice flour	Cassava flour	E.S.P*	Pumpkin powder
Protein	7.09 ^b	1.05 ^d	56.82 ^a	4.09 ^c
Fat	0.67 ^c	0.62 ^c	4.00 ^a	1.39 ^b
Ash	0.93 ^c	1.73 ^c	4.78 ^b	5.83 ^a
Fiber	1.94 ^c	1.09 ^d	2.47 ^b	2.98 ^a
Total carbohydrates	89.38 ^b	95.83 ^a	31.92 ^d	84.30 ^c

*Extruded soy protein. Data are presented as means (n = 3) & means within a row with different letters are significantly different at ($P \leq 0.05$).

Table 3. Physico-chemical analysis of flat bread on dry weight basis.

Parameters (%)	Flat bread samples				
	1	2	3	4	5
Protein	4.68 ^e	5.76 ^d	6.84 ^c	7.93 ^b	9.15 ^a
Fat	2.68 ^e	2.75 ^d	2.83 ^c	2.92 ^b	3.15 ^a
Ash	1.33 ^e	1.46 ^d	1.59 ^c	1.72 ^b	1.85 ^a
Fiber	0.57 ^e	0.78 ^d	0.91 ^c	1.05 ^b	1.18 ^a
Total carbohydrates	90.74 ^a	89.25 ^b	87.83 ^c	86.38 ^d	84.67 ^e
Carotenoids					
β -carotene ($\mu\text{g}/100\text{g}$)	102.10 ^b	465.30 ^a	467.90 ^a	481.30 ^a	484.40 ^a
Vitamin A ($\mu\text{g RAE}$) ^{**}	8.51	38.80	39.00	40.10	40.40
Caloric value (kcal/100g)	406.90	404.80	404.20	403.50	403.60
(WHC [*])	3.61 ^b	3.74 ^b	3.77 ^b	3.82 ^b	4.07 ^a

*WHC: Water Holding Capacity. **Retinol Activity Equivalent (RAE). 1 RAE = 1 μg retinol, 12 μg β -carotene, whereas the RAE for preformed vitamin A is the same as Retinol Equivalent (RE). 1 = (0 ESP), 2 = (2.5% ESP), 3 = (5% ESP), 4 = (7.5% ESP), 5 = (10% ESP). Data are presented as means (n = 3) & means within a row with different letters are significantly different at (P \leq 0.05).

from 102.10 to 484.40 $\mu\text{g}/100\text{g}$, where flat bread sample No. 5 exhibited the highest amount of β -carotene, while flat bread sample No. 1 (control) had the lowest value. This may be due to the addition of pumpkin powder during formulation **Table 1**. These results agreed with See *et al.* [39].

Vitamin A (retinol) is an essential nutrient needed in small amounts by human for the normal functioning of the visual system, growth, development and maintenance of epithelial cellular integrity, immune function and reproduction [40].

From the same **Table 3** represents vitamin A values ($\mu\text{g RAE}$) expressed as β -carotene. As vitamin A calculations dependent on β -carotene content, therefore, their values followed a similar trend than that of β -carotene. Data in the same table indicated that flat bread sample No. 5 had the highest value of vitamin A 40.40 $\mu\text{g RAE}$, while sample No. 1 had the lowest value 8.51 $\mu\text{g RAE}$. Provitamin A carotenoids are found in yellow vegetables [41].

The results in **Table 3** showed that the caloric value of flat bread nearly the same in all samples, ranged from 403.50 to 406.90 calories/100g. These results are in agreement with Haneen and Yaseen [42].

The staling of large scale manufactured flat bread may become a critical factor consideration. Bread staling is a very complex process that cannot be explained by a single effect, amylopectin retrogradation, reorganization of polymers within the amorphous region, loss of moisture content, distribution of water content between the amorphous and crystalline zone, and the crumb macroscopic structure must participate in the staling process [43] [44]. Brennan *et al.* [45] and Guarda *et al.* [46] reported that xanthan stabilized starch gels and reduced starch retrogradation. Xanthan which lead pronounced effect on viscoelastic properties yielding strengthened and gave a farinograph and extensograph curves similar to the curve of wheat flour dough.

Hence, alkaline water retention capacity (AWRC) during storage of the flat bread is the important experiment for indication on staling degree and freshness. It was determined at different periods at 0, 24, 48 and 72 h as shown in **Figure 1** it could be noticed that AWRC values of flat bread samples of 1, 2, 3, 4 and 5 at zero time were (131.6, 155.1, 164.1, 173.6 and 179.9%), respectively. After 24 h of storage sample (No. 2) had the higher (AWRC) compared with the other samples. Same trend was observed for this sample (No. 2) at 48 and 72 h. This means that the (2.5%) of ESP is suitable for production of gluten-free flat bread. These results are in agreement with Duska *et al.* [47] who mentioned that addition of ESP can be successfully used in a high quality gluten-free bread production. These results agreed with Sciarini *et al.* [7] reported that soy flours showed the best quality attributes: high specific volume, good crumb, appearance, soft texture and low staling rate. The addition of soy caused crumb softening and retarded bread staling as soy proteins had a high water holding capacity and they could interfere in starch retrogradation.

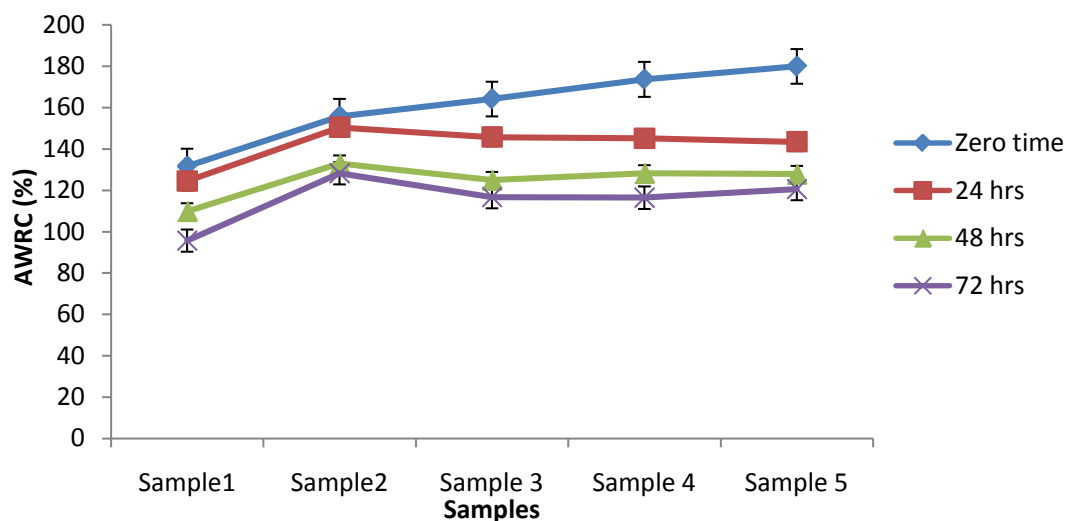


Figure 1. Alkaline water retention capacity of flat bread.

With respect to water holding capacity (WHC) it could be observed from the same **Table 3** that WHC increased insignificantly by increasing the level of ESP except for sample No. 5 which supplemented with 10% ESP. Whereas, the increasing rate in water holding capacity was 12.74%. Higher water absorption capacity of dough represents consistency which is desirable in bread making. Therefore, it is found that increase in ESP level slightly increase water holding capacity of the flat bread. These results are in agreement with Shaikh *et al.* [48] who reported that the water absorption is due to increase the quality of flour mixture which also increase the retention of moisture during dough processing for baked products.

3.2.2. Color Measurements of Flat Bread

Color is one of the most important quality attributes of flat bread [49] color measurements of the flat bread are illustrated in **Table 4**. Data indicated that supplementation with ESP and pumpkin powder significantly decreased the lightness (L) values. Control flat bread (No. 1) recorded the highest value, while flat bread No. 5 (10% ESP) had the lowest value. The redness (a) and yellowness values of the flat bread significantly increased in all flat bread samples compared with control (No. 1). Like yellowness value, color saturation value (c) of flat bread No. 5 was found to be the highest value (31.03). Besides, supplementation with ESP and pumpkin powder decreased the hue angle significantly for all samples compared with control (86.15). The results in the same table showed that the color of flat bread samples No. 1, No. 2 and No. 3 was yellow while, samples No. 4 and No. 5 was orange yellow. These results are in agreement with those reported by Lorena *et al.* [50].

3.2.3. Sensory Evaluation of Flat Bread

Photographs of flat bread are illustrated in **Figure 2**. Sensory evaluation scores for the flat bread supplemented with different levels of ESP are presented in **Table 5**. Sensory evaluation was conducted according to ranking tests which was developed for assessing the food products acceptability, in which higher the score indicates to higher acceptability and quality [42].

The results revealed that flat bread No. 2 recorded the highest score of taste (9.36) followed by sample No. 3 (9.35), whereas the lowest score found in sample No. 1 (control). Chewing ability of flat bread No. 3 recorded the highest score (9.28), followed by No. 2 (8.92). This indicates that ESP addition at 2.5 and 5% level affected the chewing ability characteristics.

From the observed data in the same **Table 5** texture score increased insignificantly in all samples compared with control. So, the addition of ESP improved the texture score. In respect the Aroma, there are no significant differences between all samples and control. Color score increased significantly for sample No. 2, No. 3 and No. 4 relative to control except for sample No. 5. Statistical analysis of sensory scores indicated that the best overall acceptability of the produced gluten-free flat bread can be obtained by 2.5 and/or 5% ESP (samples No. 2 and No. 3) in bread formulation. These results are agreement with Lorena *et al.* [50] and Kadam *et al.* [51].

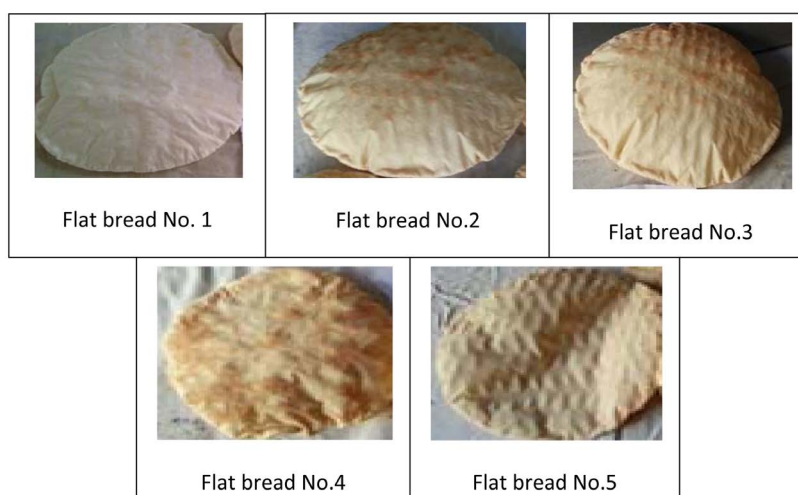


Figure 2. Flat bread photographs [1 = (0 ESP), 2 = (2.5% ESP), 3 = (5% ESP), 4 = (7.5% ESP), 5 = (10% ESP)].

Table 4. Color measurements of flat bread*.

Parameters	Flat bread samples				
	1	2	3	4	5
L	67.61 ^a	64.87 ^b	59.64 ^c	54.74 ^d	49.76 ^e
a	1.77 ^e	3.75 ^d	4.76 ^c	6.43 ^b	7.55 ^a
b	26.32 ^e	27.45 ^d	27.93 ^c	28.35 ^b	30.14 ^a
c	26.46 ^e	27.67 ^d	28.36 ^c	29.04 ^b	31.03 ^a
h	86.15 ^a	82.14 ^b	80.25 ^c	77.25 ^d	75.94 ^e
Color	Yellow	Yellow	Yellow	Orange yellow	Orange yellow

*L (lightness with L = 100 for lightness, and L = zero for darkness), a [(chromaticity on a green (-) to red (+)], b [(chromaticity on a blue (-) to yellow (+)], c (color saturation), h [(hue angle where 0° = red to purple, 90° = yellow, 180° = bluish to green and 270° = blue scale. Values are mean of three replicates ± SD, number in the same column followed by the same letter are not significantly different at 0.05 level. 1 = (0 ESP), 2 = (2.5% ESP), 3 = (5% ESP), 4 = (7.5% ESP), 5 = (10% ESP). Data are presented as means (n = 3) & Means within a row with different letters are significantly different at (P ≤ 0.05).

Table 5. Sensory evaluation of flat bread.

Parameters	Flat bread samples				
	1	2	3	4	5
Taste (10)	7.28 ^b	9.36 ^a	9.35 ^a	8.73 ^a	8.71 ^a
Chewing ability (10)	7.13 ^c	8.92 ^{ab}	9.28 ^a	8.42 ^{abc}	7.57 ^{bc}
Texture (10)	8.15 ^b	9.27 ^a	9.58 ^a	9.50 ^a	9.42 ^a
Aroma (10)	8.85 ^a	9.28 ^a	9.26 ^a	9.14 ^a	9.12 ^a
Color (10)	7.28 ^b	8.92 ^a	9.00 ^a	9.14 ^a	8.43 ^{ab}
Overall Acceptability (50)	38.69	45.75	46.47	44.93	43.25

1 = (0 ESP), 2 = (2.5% ESP), 3 = (5% ESP), 4 = (7.5% ESP), 5 = (10% ESP). Data are presented as means (n = 10) & Means within a row with different letters are significantly different at (P ≤ 0.05).

3.3. Biscuits

3.3.1. Physical Properties of Biscuits

Physical properties of biscuits such as diameter, thickness, spread ratio, weight, volume, specific volume and density were studied and given in **Table 6**. It was observed that as the level of ESP increased, the diameter, thickness, volume and specific volume of biscuits were decreased, whereas the spread ratio, weight and density of biscuits increased. The diameter, Thickness, volume and specific volume were decreased to 4.09, 0.93, 86.64 and 1.65 for sample No. 5 (20% ESP), respectively. These results are in agreement with Kumar *et al.* [52]. While spread ratio, weight, and density increased to 5.29, 49.80, and 0.63, respectively for the same sample. These results agreed with Ogunjobi and Ogunwolu [9] who found that the diameter of biscuits made from cassava and soy flour was (5.51 cm), while Onweluzo and Lwezu [53] showed that the spread ratio of biscuits supplemented with soy flour increased.

3.3.2. Chemical Analysis of Biscuits

The results in **Table 7** showed the chemical composition of biscuits. There were significant differences in all parameters considered ($P < 0.05$). The highest value for crude protein content was found in sample No. 5 (15.37%)

Table 6. Physical properties of biscuit.

Parameters	Biscuit samples				
	1	2	3	4	5
Diameter (cm)	5.11 ^a	5.07 ^{ab}	5.02 ^{ab}	5.01 ^b	4.09 ^c
Thickness (cm)	1.04 ^a	1.03 ^{ab}	1.02 ^{ab}	0.97 ^{bc}	0.93 ^c
Spread ratio	4.44 ^c	4.84 ^b	4.92 ^{ab}	4.97 ^{ab}	5.29 ^a
Weight (g)	42.20 ^b	43.40 ^b	48.10 ^a	49.10 ^a	49.80 ^a
Volume (cm ³)	108.10 ^a	105.70 ^a	100.30 ^b	97.67 ^b	86.64 ^c
Specific volume (cm ³ /g)	2.51 ^a	2.47 ^a	2.21 ^{ab}	1.91 ^{bc}	1.65 ^c
Density(g/cm ³)	0.41 ^b	0.48 ^b	0.50 ^b	0.50 ^b	0.63 ^a

1 = (0 ESP), 2 = (5% ESP), 3 = (10% ESP), 4 = (15% ESP), 5 = (20% ESP). Data are presented as means (n = 3) & means within a row with different letters are significantly different at ($P \leq 0.05$).

Table 7. Chemical analysis of biscuits on dry weight basis.

Parameters	Biscuit samples				
	1	2	3	4	5
Chemical composition (%)					
Protein	4.01 ^e	6.85 ^d	9.69 ^c	12.53 ^b	15.37 ^a
Fat	27.74 ^e	27.94 ^d	28.14 ^c	28.34 ^b	28.54 ^a
Ash	0.58 ^e	0.82 ^d	1.06 ^c	1.30 ^b	1.54 ^a
Fiber	0.55 ^e	0.67 ^d	0.80 ^c	0.92 ^b	1.04 ^a
Total carbohydrates	67.12 ^a	63.72 ^b	60.31 ^c	56.91 ^d	53.51 ^e
Carotenoids					
β -carotene ($\mu\text{g}/100\text{g}$)	865.20 ^c	1158.30 ^b	1186.50 ^b	1247.30 ^b	1386.20 ^a
Vitamin A (μg RAE) [*]	72.10	96.50	98.80	103.90	115.50
Caloric value (kcal/100g)	748.20	533.40	533.50	532.80	532.40

1 = (0 ESP), 2 = (5% ESP), 3 = (10% ESP), 4 = (15% ESP), 5 = (20% ESP). ^{*}Retinol Activity Equivalent (RAE). 1 RAE = 1 μg retinol, 12 μg β -carotene, whereas the RAE for preformed vitamin A is the same as Retinol Equivalent (RE). Data are presented as means (n = 3) & Means within a row with different letters are significantly different at ($P \leq 0.05$).

while, the lowest content found in sample No. 1 (4.01%). Also the same sample No. 5 characterized by high fat, ash and fiber except for total carbohydrates which was the lowest (28.54%, 1.54%, 1.04% and 53.51%), respectively. This may be due to the high addition level of ESP (20%).

These results are in agreement with Kumar *et al.* [52] who reported that defatted soy flour incorporation increased the nutritional status of biscuits due to high protein content. Soybean is an excellent health food and it contains 40% good quality protein, 23% carbohydrates and sufficient amounts of minerals and vitamins. Hence, it is superior to other plant proteins as it contains most of the essential amino acids except methionine, which is abundant in cereals, and it is most economical source of dietary protein. Kulkarni and Joshi [20] showed that the biscuit prepared with optimum level of replacement 2.5% (w/w) from the pumpkin powder was found to be high in carbohydrate, crude fiber, carotene and mineral matter.

The results in **Table 7** represents β -carotene $\mu\text{g}/100\text{g}$ in biscuits. It could be noticed that β -carotene content ranged from 865.20 to 1386.20 $\mu\text{g}/100\text{g}$. Biscuit samples contained higher β -carotene contents than control sample (No. 1). The highest value was 1386.20 $\mu\text{g}/100\text{g}$ for sample No. 5. These results agreed with Kulkarni and Joshi [20].

Data in the same table indicated that vitamin A (μg RAE) of biscuits expressed as β -carotene. As vitamin A calculations dependent on β -carotene content, therefore, their values followed a similar trend than that of β -carotene. It could be observed that sample No. 5 had the highest value of vitamin A 115.50 μg RAE, while sample No. 1 (control) had the lowest value 72.10 μg RAE. These results are in agreement with Booth *et al.* [54].

It could be noticed from **Table 7** that the caloric values of produced biscuits in all samples were lower than control (No. 1). This may be due to the addition of ESP. These results are in agreement with Shrestha and Noomhorm [55].

3.3.3. Color Measurements of Biscuits

Color is one of the most important quality attributes of biscuits [55]. Color measurements of biscuits are illustrated in **Table 8**. Data indicated that supplementation with ESP and pumpkin powder significantly decreased the lightness (L) values of biscuits. Sample No. 1 recorded the highest value (74.06), while sample No. 5 had the lowest (59.25). The redness (a) values increased significantly in sample No. 5 (7.87) compared with No. 1 (2.45). Regarding yellowness (b) values, supplementation with ESP significantly increased the yellowness of biscuits, whereas, sample No. 5 recorded the maximal b value, in contrast, sample No. 1 recorded the minimal b value. Like yellowness value, color saturation value (c) of the same sample No. 5 was found to be the highest value (31.76). In contrast, the same sample had the lowest hue angle (h) value (75.63), compared with control sample (84.86). The resultant in **Table 8** showed that the color of samples No. 1 and No. 2 was yellow, while samples No. 3, No. 4 and No. 5 was Orange yellow. These results are in agreement with those reported by Pereira *et al.* [56].

3.3.4. Texture Profile Analysis of Biscuits (TPA)

Texture analysis is primarily concerned with measurement of the mechanical properties of a product, often a

Table 8. Color measurements of biscuits*.

Parameters	Biscuit samples				
	1	2	3	4	5
L	74.06 ^a	71.66 ^b	67.43 ^c	64.74 ^d	59.25 ^e
a	2.45 ^e	3.71 ^d	5.17 ^c	6.84 ^b	7.87 ^a
b	26.84 ^e	27.46 ^d	28.15 ^c	28.64 ^b	30.77 ^a
c	26.98 ^e	27.73 ^d	28.56 ^c	29.45 ^b	31.76 ^a
h	84.86 ^a	82.26 ^b	78.63 ^c	76.53 ^d	75.63 ^e
Color	Yellow	Yellow	Orange yellow	Orange yellow	Orange yellow

*L (lightness with L = 100 for lightness, and L = zero for darkness), a [(chromaticity on a green (-) to red (+)], b [(chromaticity on a blue (-) to yellow (+)], c (color saturation), h [(hue angle where 0° = red to purple, 90° = yellow, 180° = bluish to green and 270° = blue scale. Values are mean of three replicates \pm SD, number in the same column followed by the same letter are not significantly different at 0.05 level. 1 = (0 ESP), 2 = (5% ESP), 3 = (10% ESP), 4 = (15% ESP), 5 = (20% ESP); Data are presented as means (n = 3) & Means within a row with different letters are significantly different at (P \leq 0.05).

food product, as they relate to its sensory properties detected by human via applying controlled forces to the product and recording its response in the form of force, deformation and time. Texture measurements can be very valuable for the quality control and process optimization as well as for the development of new products with desirable properties and characteristics [57].

Data in **Table 9** presented the textural parameters assessed from texture profile analysis (TPA) test curves results for the biscuits samples. A marked increase in hardness from 2933 g to 4098 g was observed. On the other hand, the biscuits become harder with increasing ESP level. Data showed that sample No. 5 had the highest hardness value (4098 g) compared to other samples and control (2933 g). This may be due to the effect of ESP and pumpkin powder in formulation. These results agreed with Kulkarni and Joshi [20] who reported that the replacing 2.5% of formulation by pumpkin powder, the hardness of the biscuits increased compared control sample. Hoojjat and Zabik [58] and Lee and Beuchat [59] reported that more strength was needed to break cookies incorporated with legumes flour. This might have resulted from incorporation of protein rich flour which need more water to obtain good cookie dough, and the cookies prepared from high-absorption dough tend to be extremely hard.

A similar trend was observed for adhesiveness as it considered maximum negative force generated during probe return. The results in **Table 9** showed that biscuit sample No. 5 had the highest value ($5.0 \text{ g}\cdot\text{s}^{-1}$) compared with sample No. 1 ($1.0 \text{ g}\cdot\text{s}^{-1}$). In conclusion increment of hardness and adhesiveness may be due to adding ESP and pumpkin powder.

3.3.5. Sensory Evaluation of Biscuits

Sensory evaluation is considered to be a valuable tool in solving problems involving food acceptability. It is useful in product improvement, quality maintenance and more important in a new products development [60].

Photographs of biscuits are illustrated in **Figure 3**. Sensory evaluation of biscuit samples are shown in **Table 10**. It was observed that there were insignificant differences in appearance, color, odor and taste for all samples. While, there was significant differences between all samples and control for texture parameter. Addition of ESP at 20% (sample No. 5) significantly decreased the texture compared to the other samples. From the observation data in **Table 10** it could be concluded that, the best addition level to obtain high overall acceptability score was 5% ESP.

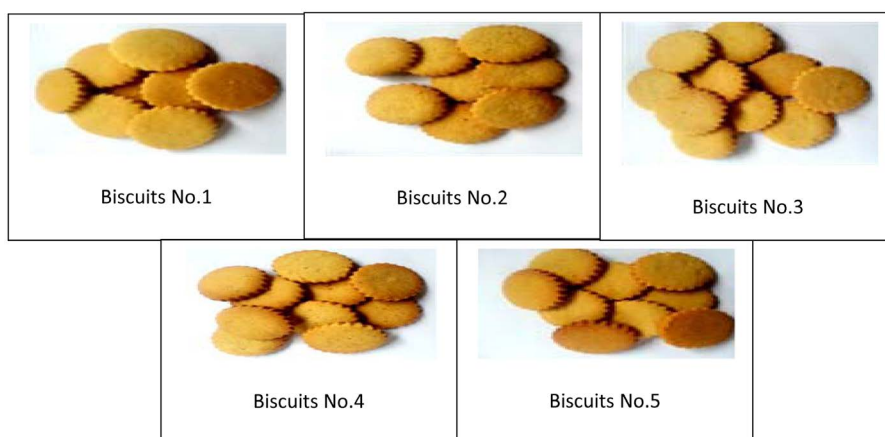


Figure 3. Biscuit photographs [1 = (0 ESP); 2 = (5% ESP); 3 = (10% ESP); 4 = (15% ESP); 5 = (20% ESP)].

Table 9. Texture properties of biscuits.

Parameters	Biscuit samples				
	1	2	3	4	5
Hardness (g)	2933	3234	3612	3806	4098
Adhesiveness ($\text{g}\cdot\text{s}^{-1}$)	1.0	2.0	2.0	3.0	5.0

1 = (0.0 ESP), 2 = (5% ESP), 3 = (10% ESP), 4 = (15% ESP), 5 = (20% ESP).

Table 10. Sensory evaluation of biscuits.

Parameters	Biscuit samples				
	1	2	3	4	5
Appearance(10)	9.45 ^a	8.91 ^b	8.42 ^b	8.85 ^{ab}	8.65 ^{ab}
Color (10)	9.64 ^a	9.25 ^{ab}	8.55 ^b	8.51 ^b	8.71 ^{ab}
Odor (10)	9.53 ^a	9.11 ^a	9.12 ^a	8.95 ^a	8.82 ^a
Texture (10)	9.51 ^a	7.92 ^b	7.65 ^b	6.75 ^c	6.61 ^c
Taste (10)	9.35 ^a	9.13 ^{ab}	8.85 ^{ab}	8.45 ^{ab}	8.35 ^b
Overall acceptability (50)	47.48	44.32	42.59	41.51	41.14

1 = (0.0 ESP), 2 = (5% ESP), 3 = (10% ESP), 4 = (15% ESP), 5 = (20% ESP). Data are presented as means (n = 10) & means within a row with different letters are significantly different at ($P \leq 0.05$).

4. Conclusion

Celiac disease is an autoimmune disorder characterized by intolerance to gluten. It is considered to be a potentially significant cause of poor health in population. All ingredients under study influenced the flat bread and biscuits quality. The importance of added extracted soy protein (ESP), pumpkin powder, rice flour and xanthan gum to cassava flour was evident in assessment of the parameters: nutritional value, texture, color and organoleptic acceptability which improved as these ingredients added. The optimized additions of ESP were 2.5% and 5% for flat bread and 5% for biscuits. The optimal ratio of cassava and rice flour was 1:1 (w/w), 0.1% xanthan gum and 7% pumpkin powder. Addition of xanthan gum helps in formulation of dough. It acts like wheat gluten. On the other hand, pumpkin powder improved the nutritional value of the produced flat bread and biscuits with attractive color to the products. Thus, the produced flat bread and biscuits are suitable for those people suffering from celiac disease especially for children with protein and vitamins deficiency. Addition of ESP not only enhanced the nutritional value of flat bread but also retarded the staling. This study succeeded to achieve flat bread and biscuits nearly with high quality and acceptability.

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