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Improving the Nutritive Value and Quality Parameters of Gluten Free Bread by Using Natural and Economical Ingredients

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Abstract Absence of gluten in gluten free bread is an important technological challenge, in this study sweet whey protein powder and fresh yogurt were added individually and together at 10 and 15 %. (W/W corn flour). Different levels of yogurt and whey protein powder were incorporated into gluten-free bread formulations. Bread quality parameters i.e. chemical, physical, textural, staling rate, water activity, sensory and economical evaluation were performed to assess the nutritional and technological qualities of the free gluten bread. Results confirmed that the yogurt and whey protein addition led to a significant increase in protein, fat, ash, crude fibres, calories, Ce, Fe and Zn content in bread while significant decrease in available carbohydrates comparing with the control. Textural profile analysis showed a decrease in hardness, gumminess and chewiness when whey protein and yogurt were added to bread formula either they added individually or together. The staling rate indicated that whey protein and fresh yogurt caused retarding in the staling rate and the whey protein bread can be kept up to 3 days with good freshness. Whey protein or yogurt addition to bread formula show a significant increase in appearance, crust colour, taste, texture, layer separation and overall score. Rising levels of supplementation with whey protein and yogurt generally caused an increase in all sensory scores of free gluten bread comparing with the control, this improvement was noticed when whey protein and yogurt were added together. Water content and water activity measurements was in the allowance range, so that water activity predicted the stability and keeping safety and quality of the prepared bread. The gluten - free bread was safe for up to 3 days for consumer. Whey protein and fresh yogurt specially when they added together improving the daily diet of celiac people, so whey protein bread and yogurt bread enhanced the nutritional value of gluten free bread such as protein, Ca, Fe and Zn, also it had good efficiency of covering the recommended daily allowances (RDA %). The cost of fortified bread with whey protein and yogurt was increased by 9.84% to 44.09 % as compared with the control cost, but that increase in the cost can be adjusted to consumer by health benefits.

Keywords: *gluten-free bread, yogurt, whey protein*

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1. Introduction

The celiac disease caused by the ingestion of gluten in genetically susceptible individuals and it is effect about 1-3% of the population worldwide [1].

There has been a huge increment of studies to improve the functional and dietary properties of gluten free products [2] and [3]. However, different investigations expressed that the consumers stay unsatisfied with the quality of the gluten free items in the market, centring attention to their inadequacy on overall appearance and nutritional values, compared to the gluten-containing counterparts [4].

Absence of gluten in bakery goods is an important technological challenge, gluten-free breads (tortilla) with low functional and nutritional properties. However, these issues can be limited by utilizing new protein sources, by the addition of nutritional added-value products. Gluten-free products, particularly breads being essentially founded on refined flours and starches, are large described by poor quality credits, including dry, crumbling texture, color, and mouth feel and undergo fast staling [5] and [6].

Yogurt is considered as the most popular dairy product worldwide for its nutritional and health benefits. It is a source of protein (casein), polysaccharides, vitamins (B2, B6, and B12), and minerals (Ca, P, and K). Also, yogurt is an interesting alternative for new bakery products [7] and [8].

In addition to, yogurt in gluten-free bread formulations can be a fascinating way to utilize the gluten network with enhancing the nutritional value of gluten-free breads.

The large amounts of whey protein produced all the years by the cheese production, whey protein contains high organic matter such as protein and lactose meanwhile, whey protein is considered an important pollution problem and several strategies have been developed to utilize this whey protein (by-product) including bringing it back to the food value-chain, as in circular economy principles [9] and [10].

Whey is classified to two types, sweet whey with a pH of about 6.02 to 6.58 and acid whey with a pH of 3.57 to 4.341 [11].

Many studies concentrated on the application of whey and whey powders in food industry [12].

Whey proteins are high-quality proteins with a high biological value and contain high levels of amino acids, such as leucine, isoleucine and valine, which they are lower in other plant-based proteins sometimes used in bakery products [13].

Past studies showed the impact of whey protein and yogurt on dough behaviour utilizing the rheological tests however the aim of this research was to explore the potential benefit of powder whey protein and fresh yogurt addition on free gluten bread to overcome the technological challenge involved in free gluten with improving the nutritional value of gluten free bread.

Different levels of sweet powder whey protein and fresh yogurt addition individually or together to free gluten dough formulation. The free gluten bread quality parameters such as nutritional quality, texture, staling, and sensory characteristics were evaluated. The results indicated that the bread quality, sensory properties and nutritional profile were significantly improved by adding fresh yogurt and powder whey protein especially, when they are added together.

2. Materials and Methods

-Yellow corn flour 97 % was obtained from Egyptian Company for maize products 10th Ramadan City, Egypt.

-Powder whey protein, yogurt, dry yeast, corn oil, salt were obtained from local super market, Giza, Egypt.

Preparation of flat free gluten bread

-Gluten free bread was prepared as mentioned by [14] with some modification, the formula is shown in Table 1.

Table 1. The formula of gluten free bread

Sample	Control	1	2	3	4	5	6
Corn flour(g)	1000	1000	1000	1000	1000	1000	1000
Powder whey protein (g)	-	100	150	-	-	100	150
Fresh yogurt (g)	-	-	-	100	150	100	150
Corn oil (ml)	50	50	50	50	50	50	50
Dry yeast (g)	10	10	10	10	10	10	10
Salt (g)	10	10	10	10	10	10	10
Boiling water (ml)	920	925	927	910	905	910	910

Processing of free gluten bread:

Gelatinization of corn flour: corn flour (one kg) was mixed with powder whey protein or fresh yogurt in boiling water and heated on hotplate for 15 min. with continuous mixing [14].

Processing method: Corn flour was mixed with dry yeast, corn oil and salt. Powder whey protein and fresh yogurt were incorporated in the dough as mentioned in Table 1.

Boiling tap water heated for 15 min. with continuous mixing. The dough was left for 15 min. at 30Ċ and 85 % relative humidity then, the dough was divided into 25 g and flattens at thickness 3mm and fermented for 25 min. at 30°C with 85 % relative humidity, then baked at 350-400°C for 1-1.25 min. bread loaves were allowed to cool before scoring.

Physical characteristics of and flat free gluten bread):

Textural analysis of free gluten bread: hardness, cohesiveness, adhesiveness, gumminess and chewiness of cup cakes were measured using Brookfield Engineering Lab. Inc., Middleboro, MA. 02346-1031. USA based on [15]. method 74-09.

Water activity (aw):

The water activity (aw) of free gluten bread was measured using Rotronic Hygrolab 3 CH-8303, Switzerland as measured by [16].

Determination of bread staling: after baking the bread was cooled at room temperature Alkaline Water Retention Capacity (AWRC)as indicate on staling was determined at zero time and after 12, 24, 48 and 72 hr of storage according to the method of [17]. The bread was cut into small pieces, dried at 50 C0 then grounded to pass 60 mesh sieve. Five grams of the ground bread were placed into dry centrifuge tube and 25 ml of NaHCO3 solution (8.4 gm sodium bicarbonate dissolved in one litter water were added and the tube was stoppered and shaked until wet. Then left for 20 min with shaking each 5 min. The contents were centrifuged at 2500 rpm for 15 min. And supernatant was decanted. The experiment was duplicated and average gain of the 2 runs was multiplied by 20 to give AWRC % and the rate of AWRC decrease % (RD%) was calculated using the following equation.

$$RD\% = AWRC\%(0\ time) - AWRC\%(n\ time) / AWRC(0\ time)$$

Sensory evaluate on of free gluten bread:

Sensory evaluation was determined and scoring scheme was established as mentioned by (18), general appearance, crust color, texture, taste, odor, roundness, layer separation , flavor and over all scores.

Chemical analysis for raw materials flat free gluten bread:

- Moisture, fat, ash, crude fibre and crude protein and essential minerals were determined for according to [19].

-Available carbohydrate was calculated by difference, 100-(protein + fat +fiber + ash) as mentioned by [20].

-Total calories were calculated according to [21] as follows:

$$E = 4X\left(Carbohydrate\% + protein\%\right) + 9X\left(fat\%\right).$$

Statistical analysis:

The obtained results for chemical composition and sensory evaluation were statistically analyzed by the least significant difference value (LSD) at 0.05 levels probability by [22].

3. Results and Discussion

The chemical composition of corn flour, powder whey protein and fresh yogurt (on dry weight basis) are presented in Table 2 whey protein and fresh yogurt had considerable amount of protein, fat and ash. Meanwhile corn flour had the least amount of protein, fat and ash. Corn flour showed the highest component of carbohydrate. These results agreed with [4,10,14].

Regarding the minerals profile, yogurt was the highest value of Ca then the whey protein. Zinc was nearly amount in whey protein and yogurt, meanwhile corn flour had the highest value of Fe and Zn compared with the other raw materials.

4. Nutritional Quality of the Free Gluten Bread

Table 3 exhibits the nutritional values of gluten free bread on dry weight basis. Data proved that the protein, fat, ash and fiber were increased significantly with increasing the level of whey protein and yogurt addition compared to the control bread. The significant increase in protein was pronounced more in samples 5 and 6 which contained powder whey protein and yogurt together. Fat significantly increased in all supplemented with yogurt and whey protein comparing with control. Crude fiber ranged between 2.28 to 3.71in all supplemented samples, meanwhile control bread contained 1.62 % of crud fiber. Ash content was increased significantly in all supplemented samples, this may be due to the high content of minerals in yogurt and whey protein. It could be noticed that the whey protein bread had high value of protein, ash and fiber comparing with yogurt bread when we used whey protein or yogurt individually, this increment may be due to the high protein and salts content in whey protein than that in the yogurt. These results agreed with [4] and [10] who found the yogurt and whey protein caused an increase in protein, fat, ash and fiber content in supplemented bread. Considering, total carbohydrate, it decreased significantly in supplemented bread compared to control sample. Meanwhile calories were increased significantly comparing with the control.

Table 4 represents the important minerals for growth, Ca, Fe and Zn content mg/100g bread. Fe serves as carrier of oxygen to the tissues and as an integrated part of important enzyme in various tissues. Zn is essential for normal growth and development of the immune response and participating as a cofactor for more than 300 enzymes.

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

Sample	Protein %	Fat %	Ash	Fiber %	A. C. %	Ca Mg/100g	Fe mg/100g	Zn mg/100g
Corn flour 97%	9.55±	2.23±	1.21	1.41	85.60	51.89±0.02c	1.43±0.02a	1.56±0.09a
Powder whey protein	38.57±	12.71±	4.54	4.32	39.86	105.70±0.02b	0.08±0.01b	0.42±0.03b
Fresh yogurt	35.22±	32.41±	4.91	5.11	22.35	122.98±0.02a	0.05±0.01c	0.52±0.01c
LSD 5%						0.03	0.02	0.08

A.C.: Available carbohydrates.

Table 3. Nutritional values and mineral profile for gluten free bread (on dry weight basis)

Sample	Protein%	Fat %	Ash%	Fiber%	AC%	Calories kcal/100g
Control	9.11 ± 0.1^{d}	5.2 ± 0.2^{c}	2.3 ± 0.3^{d}	1.62 ± 0.2^{d}	81.67 ± 1.0^{a}	410 ± 10^{f}
1	13.23 ± 0.23^{c}	6.71 ± 0.2^{d}	2.84 ± 0.2^{bc}	2.30 ± 0.3^{bc}	74.92 ± 0.5^{b}	412.99 ± 10^{c}
2	15.04 ± 0.5^{b}	7.42 ± 0.2^{c}	3.11 ± 0.11^{b}	2.63 ± 0.2^{b}	71.8 ± 1.0^{c}	414.14 ± 0.1^{d}
3	13.03 ± 0.5^{c}	8.2 ± 0.2^{b}	2.37 ± 0.2^{bc}	2.28 ± 0.1^{bc}	73.18 ± 0.5^{b}	$42,.96\pm0.5^{b}$
4	14.82 ± 0.5^{b}	9.72 ± 0.3^{b}	2.97 ± 0.1^{bc}	2.61 ± 0.2^{b}	69.88 ± 1.0^{d}	426.28 ± 0.5^{a}.
5	15.21 ± 0.2^{b}	8.31 ± 0.3^{b}	3.31 ± 0.3^{b}	2.91 ± 0.2^{b}	70.26 ± 0.5^{d}	416.67 ± 0.5^{c}
6	17.91 ± 0.5^{a}	9.51 ± 0.5^{a}	3.89 ± 0.1^{a}	3.71 ± 0.2^{a}	64.98 ± 1.0^{c}	417.15 ± 0.5^{c}
LSD	0.64	0.44	0.33	0.33	1.32	1.0

C.: available carbohydrates.

Table 4. Mineral content of gluten free bread (mg/100g)

Sample	Fe	Zn	Ca
control	1.32±0.02c	1.31±0.01d	39.8±0.09g
1	1.44±0.02b	1.35±0.02bc	50.11±0.11f
2	1.48±0.1b	1.37±0.02bc	55.31±0.01d
3	1.41±0.02bc	1.38±0.02b	52.31±0.1e
4	1.45±0.02b	1.41±0.01b	57.42±0.1c
5	1.53±0.1b	1.43±0.03b	61.8±0.1b
6	1.64±0.01a	1.53±0.01a	72.8±0.2a
LSD	0.09	0.03	0.18

Data revealed that, whey protein and yogurt caused a significant increase in Ca, Fe and Zn in supplemented bread. So that, bread samples which fortified with either yogurt or whey protein had higher content of Ca, Fe and Zn comparing to control. Ca, Fe and Zn were increased in gluten free bread by increasing the amount of yogurt and whey protein. It could be noticed that the yogurt caused higher increment for Ca and Zn comparing with the whey protein addition. This increment in minerals in fortified samples as a result to the bread formulas, yogurt and whey protein are rich in the important minerals. The fortified bread has considerable amount of Ca, Fe and Zn when yogurt and whey protein added together. So that the fortified samples were preferred for celiac disease people. These findings are in the same findings with [4] and [23] who reviewed the nutritional quality of yogurt and whey protein and their effects to add nutritional value of bread.

Water content and water activity (aw) of gluten free bread

Water is an important substance present in all foods, which contributes to food texture, structure stability and overall quality. Its content is a very important factor controlling the rate of food deterioration which in turn has an additional effect on the shelf life of intermediate - moisture foodstuffs [24].

Table 5 represents the water content and water activity of gluten free bread. Data revealed that samples 5 and 6 recorded high water content (30.1 % and 30%) respectively. This may be due to the highest fat content which prevents the moisture uptake. In contrast sample 1 recorded the minimal water content 29.11 %.

Water activity (wa) is a measurement of the availability of water for biological growth. It has already been identified as an intrinsic factor in determining the safety or shelf -life of a product.

Data proved that control bread recorded the highest aw (0.91) followed by sample 4 and 5 (0.89). In contrast sample no 1 recorded (0.8). This may be related to their water content. According the water activity value of all samples was considerably low. (25) stated that aw is a particularly important factor influencing spoilage of many bakery products such as breads and cakes has levels above 0.94.

Table 5. Water content and water activity (aw) of gluten free dread

Sample	Water content %	Water activity (aw)
Control	29.51±1.0a	0.90±0.1a
1	29.11±1.0a	0.80±0.0b
2	29.51±0.5a	0.83±0.0b
3	29.80±0.5a	0.88±0.0a
4	29.82±0.5a	0.89±0.1a
5	30.11±0.1a	0.89±0.01a
6	30.0±0.3a	0.83±0.01b
LSD 5%	1.19	0.09

Sensory evaluation of gluten free bread:

The sensory characteristics evaluated for gluten free bread with whey protein and fresh yogurt individually and whey protein and yogurt blends of them compared to the control. Data in Table 6 showed that, addition of whey protein and yogurt individually or together caused a significant improving in sensory characteristics i.e. appearance, crust color, taste, texture, layer separation, odor and overall score comparing with the control. This result was confirmed with [4]. Addition of whey protein and yogurt together have more good effect on sensory characteristics of bread than we used yogurt or whey protein individually. So that, fortified samples no 5 and 6 were highly accepted (very good). This may be the good effect of yogurt and whey protein on sensory characteristics especially on taste, appereance and color of bread.

Table 6. Sensory quality attributes of free gluten bread

	Appearance	Crust	Taste	Texture	Layer separation	0d0r	Roundness	Overall
Control	15.5 ±1.0^{c}	12.5 ±0.5^{c}	15±1.0^{c}	8±1.0^{ab}	10.5±1.0^{c}	9.5±0.5^{b}	9±1.0^{a}	80±1.0^{f}
1	18.4±1.1^{ab}	13.3±0.3^{ab}	19.2±0.2^{ab}	8.5±1.0^{ab}	12.9±1.0^{ab}	9.9±0.1^{a}	9.0±1.0^{a}	91.2±1.0^{e}
2	18.5±1.0^{ab}	13.5±0.3^{ab}	19.3±0.3^{a}	8.5±0.5^{ab}	13.5±0.5	9.9±0.1^{a}	9.8±0.2^{a}	93±0.2^{d}
3	19.5±0.5^{a}	14±0.5^{b}	19.5±0.5^{a}	9±1.0^{a}	13.5±1.0^{a}	10±0.0^{a}	9.5±0.5^{a}	95±1.0^{c}
4	19.5±0.5^{a}	14.5±0.5^{a}	19.5±0.5^{a}	9.5±0.5^{a}	14.5±0.5^{a}	10±0.0^{a}	9.5±0.5^{a}	97±1.0^{ab}
5	19.5±0.5^{a}	14.5±0.5^{a}	20±0.0^{a}	10±0.0^{a}	14.5±0.5^{a}	10±0.0^{a}	9.6±0.5^{a}	98±1.0^{a}
6	20±0.0^{a}	15±0.0^{a}	20±0.0^{a}	10±0.0^{a}	14.5±0.5^{a}	10±0.0^{a}	10±0.0^{a}	99.5±0.5^{a}
LSD	1.18	0.7	0.77	1.13	1.21	0.3	1.01	1.42

Table 7 presents texture profile analysis results for gluten free bread, it could be observed that a decrease in hardness of bread supplemented with yogurt and whey protein. The hardness showed that the control bread became harder compared to other supplemented bread. Yogurt and whey protein addition had a positive impact to decrease hardness. They improve texture properties and such effects are related to they ability to bind water and retain moisture retarding the starch crystallization and decrease the bread hardness as a result of the polysaccharides of yogurt and whey protein as reported by [26].

Adhesiveness of gluten free bread was decreased as hardness decreased .

The similar relation between hardness, adhesiveness and resilience.

Table 7. texture properties of gluten free bread samples.

Sample	Hardness (N)	Adhesiveness (Mj)	resilience
Control	17.94	2.2	0.26
1	11.4	1.1	0.12
2	8.53	1.0	0.11
3	7.57	0.94	0.12
4	6.37	0.92	0.10
5	9.18	0.91	0.12
6	8.82	0.91	0.11

Table 8. Alkaline water retention capacity (AWRC) of gluten bread supplemented with whey protein and yogurt

sample	AWRC% At zero time	AWRC% Afte12 hr	RD%	AWRC% After 24 hr	RD%	AWRC% After48 hr	RD%	AWRC %after72 hr	RD%
control	410.21	320.21	21.94	299.51	26.99	299.51	26.99	280.11	36.59
1	450.23	415.23	70.77	395.21	12.22	395.21	12.22	355.23	21.1
2	467.51	437.51	6.42	415.51	11.12	415.51	11.12	380.0	18.72
3	470.23	445.71	5.21	428.11	9.16	425.51	9.51	397.11	15.54
4	482.14	463.14	3.94	437.00	9.3	437.31	9.2	413.51	14.23
5	475.23	457.32	3.77	447.00	9.3	445.1	6.34	425.31	10.50
6	491.41	481.41	2.03	448.50	7.98	455.31	7.30	437.21	11.00

Staling evaluation of gluten free bread:

The rate of staling of bread as affected by addition of whey protein and fresh yogurt was evaluated by alkaline water retention capacity (AWRC)determination after 0, 12, 24, 48 and 72 hrs of baking as presented in Table 8.

Results showed that the rate of AWRC decrease (RD) was affected by addition of either whey protein and fresh yogurt at different levels as compared to the control. Addition of either whey protein or yogurt individually or together caused an increase in AWRC meanwhile RD was decreased, hence retarded the staling compared to the control after 0, 12, 24, 48 and 72 hrs of baking. These results may be due to increase fat and protein in whey protein bread and yogurt bread. These results agreed with those reported by [4] and [27] who found that the reduction in rate of staling might be due to the high protein and fat content.

Percentages of the recommended daily allowances (RDA%) are provided from gluten free bread.

Multiple micronutrient (MMN) deficiencies often occur simultaneously as a result of a poor quality diet. The percentages of the recommended dietary allowances (RDA%) provided from 100 g of gluten free bread for children 4-8 years are shown in Table 9. It could be observed that the values of RDA % for protein, minerals (iron, calcium and zinc) were improved in either whey and yogurt bread especially when whey protein and yogurt were added together.

Table 9. The percentages of the recommended dietary allowances (RDA%) are provided from 100g gluten free bread of some nutrients for children (4-8 years)

Sample	RDA* protein 19g	RDA* Fe 10mg	RDA Ca800mg	RDA Zn 5mg
Control	47.95	13.2	4.98	26.2
1	69.63	14.4	6.26	27.0
2	79.16	14.8	6.91	27.4
3	68.58	14.1	6.54	27.6
4	78.0	14.5	7.18	28.2
5	80.05	15.3	7.73	28.6
6	94.26	16.4	9.1	30.6

RDA*= value of nutrient in reference.
RDA %= value of nutrient in sample /RDA for the same nutrient in reference X100.
RDA was determined according to [28].

The economic evaluation of gluten free bread:

The cost of gluten free bread was increased with increasing the level of whey protein and yogurt.

The percentage of increasing in the cost compared to the control as mentioned in Table 10.

Table 10.

Sample	The increasing percentage of the cost %
control	-
1	9.86
2	12.68
3	25.63
4	36.62
5	40.31
6	44.09

5. Conclusions

Gluten-free bread formulations, with different levels 10 and 15 % (w/w corn flour), of whey protein and yogurt addition individually or together were evaluated by nutritional quality, physical, textural characteristics, staling measurements and sensory evaluation.

The results of the present work showed that the functionality of gluten-free breads, in terms of bread making performances, quality parameters, and nutritional profile can be successfully improved by the addition of fresh yogurt and whey protein especially when whey protein and yogurt were added together.

Resuming, the yogurt and whey protein addition showed to be a potential ingredient to improve the quality of gluten-free breads, resulting in softer breads with less hardness, chewiness and lower staling rate, compared to control bread.

Related to the nutritional composition, the addition of yogurt and whey protein revealed to be an attractive ingredient to enhance the nutritional value of gluten free breads, increasing protein, fat, ash, fiber, calories and mineral contents i.e Ca, Fe and Zn meanwhile a little reducing the carbohydrates intake. That fortification by whey protein and yogurt considered a good contribution to improve the daily diet with covering the recommended daily allowance (RDA%) for the celiac people.

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