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Nutritional, Physicochemical and Organoleptic Properties of Cookies Enriched with Green Lentil Dietary Flour Proposed for Children

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Abstract

The aim of this study was to partially determine the nutritional and functional properties of cookies enriched with green lentil dietary flour proposed for children. The proposed cookies were enriched with lentil dietary flour at different concentrations, at the expense of the proportion of wheat flour. The cookies were divided into three different groups based on different formulas, the first group was without green lentil (control), while the other two groups were enriched cookies with green lentil flour "6%" and "12%". Results of protein analysis showed that cookies with 6% lentil flour had the highest protein content. Results of protein solubility indicated that cookies fortified with green lentil flour had the highest extraction values in comparison to the control samples. The molecular weight of proteins from ingredients (lentils, wheat, skimmed milk, whey protein, and egg), extracted by high ionic strength buffers, remained unchanged. The combination of ingredients normally impacts a change in proteins, but the major proteins preserve their nutritional characteristics even after being mixed in different formulas. The results showed that the dry matter and ash contents of cookie samples increased by the addition of lentil dietary flour. Colour measurement results showed that the control samples had the highest L^* values, while samples of 6% had the highest a^* and b^* values. Sensory analysis results indicated that the utilization of lentil flour in the formula resulted in acceptable quality characteristics in both cookie types (6 and 12%). Overall, data indicates cookies enriched with green lentil dietary flour have fortified their protein and phytochemical contents. The utilization of protein powder derived from lentils may enhance the nutritional quality and functional properties of cookies that can be proposed for school and pre-school kids.

Keywords: *Children Diet and Nutrition; Green Lentil; Protein Flour; Fortified Cookie; Kids Food*

Abbreviations

WE: Weber-Edsall; WSP: Water Soluble Protein; PB: Phosphate Buffer; SDS-PAGE: Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis

Introduction

A basic healthy diet in early infancy and childhood is crucial in determining a child's healthy development and impacts overall health later in the life cycle. In the puberty period, numerous significant lifestyle choices are made, for instance regarding particular nutritional behaviour which may be repeated later in life and may determine the state of health [1]. Malnutrition contributes directly or indirectly to more than 60% of 10 million child deaths each year [2]. For instance, between 44% and 87% of school-aged boys and girls do not meet their recommended intake for calcium [3]. Far too many of our children and young people are not getting the diets they need, which is undermining their capacity to grow, develop and learn to their full potential [4]. Globally, at least 1 in 3 children under 5 is not growing well due to malnutrition in its more visible forms: stunting, wasting and overweight. While 50% of children under 5 suffer from hidden hunger due to deficiencies in vitamins and other essential nutrients [4]. Stunting is a health problem affecting children at the peak of their linear growth. Its prevalence is decreasing in developed countries, but in some African countries the incidence still increases. This health problem usually appears in children that have stopped exclusive breast-feeding and now on external food. Causes of stunting are usually nutritional, and as a result management is mainly by adding high energy food, macronutrients and micronutrients (zinc, vitamin A) to increase the quality of ingested food [5]. Despite the decreasing trend in malnutrition over the past three decades, following the COVID-19 pandemic and its economic consequences, an increase in the prevalence of malnutrition is plausible [6]. From 2000 to 2016, the proportion of overweight children (5 to 19 years old) rose from 1 in 10 to almost 1 in 5 [3]. Unfortunately, children prefer to eat sweets or fast food to the detriment of healthy food like vegetables and fruits [7]. Among which are the biscuits, cookies and other snacks that are highly loaded with refined sugars and corn syrups that are high in glycemic index. Usually, such snacks lack many nutrients like vitamins, proteins, and functional phytochemicals and nutraceuticals. Multiple intervention strategies based on risk factors can reduce early malnutrition of children under the age of five [8].

Recently, researchers have been focusing on functional foods, which are foods that are given an additional function (often one related to health-promotion or disease prevention) by adding new ingredients or more of existing ingredients [9]. Protein-containing foods should be consumed during the first stage of the development of muscle, brain, immune system and bone in children. Food of animal origin, including dairy products, meat, and eggs are mainly foods with a high protein content. Additionally, consumption of plant food rich in proteins is also important in the early stages of the life cycle, particularly legumes and beans. Because these foods contain essential amino acids and complete proteins. They must be on a children's diet. However, children who are fed with food lacking protein usually face many health problems. For example, Kwashiorkor and Marasmus diseases usually occur due to protein deficiency.

Legumes, commonly referred to as "pulses", are grown in all parts of the world and are typically marketed as dry products. Pulses are grain legumes and include lentils, peas, chickpeas, and beans, which are rich in protein and starch [10]. Grain legumes have significant cultural and historical importance as a staple food. They are essential to human diets in many parts of the world. Pulses are unique for a human diet in terms of their nutritional profile. They are rich in protein (15.9 to 31.4 g/100 g dry matter), carbohydrates, dietary fibre, some minerals and vitamins, and they are low in fat. Research has indicated that consumption of pulses may have potential health benefits including reduced risk of cardiovascular disease, cancer, type-2 diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal disease and reduction of LDL cholesterol [11].

Lentil is an important high protein pulse crop, botanically classified as Lens culinaris [12]. Lentils are most common in the regions of the Middle East and Asia and form the basis of the diet for the people living in these areas. India, the 2^{nd} largest producer of lentils following Canada, which accounts for total production of 1.31 million metric tons in the year 2013 [12]. Canada's lentil production reached 674,000 tonnes in the year 2007, placing Canada as one of the largest producers in the world. Despite this, Canadian lentil consumption remains slow (i.e. 0.6 kg/capita/year) [13].

Lentils are a key source of protein for the human body, particularly in parts of the world where meat and milk consumption is constrained by factors such as low availability, ethical reasons or allergenicity. Some researchers suggest that legume-based products are essential in our daily diet for leading a healthy life [14]. Lentil contains high amounts of protein, fibre, vitamins (e.g. folate) and minerals and is low in fat.

Childhood nutrition, if not given serious attention, will lead to various health risks as children mature. When it comes to the 'first food choice for kids' (or complementary foods), they should start consuming healthy processed food at around the age of 3 years. It should concern us all that so many children around the world suffer from eating unhealthy snacks. This situation demands a determined and effective policy response- a response that can only come about if there is industrial will to protect and respect children's nutrition rights, notably the right to adequate healthy processed food. Safeguarding this right requires the food producers and governmental bodies to implement functional food to fortify the conventional food snacks. Therefore, everyone ensures that every child has access to food that, at the very least, is nutritional, palatable, healthier and is culturally appropriate and safe to minimize the risk of common diseases caused by improper food habits and lifestyles (empty-calorie food). These trends should redirect food industry priorities, shifting efforts to providing drastically better healthy food and better access to functional snacks. In summary, green lentil is a good source of minerals, valuable proteins and functional components, and its dietary flour has the potential to maintain children's health if implemented properly in snacks.

Therefore, this research article aimed to fortify a kids' cookie that is heavily consumed by kids at an early stage of their life cycle. The research focuses on the enrichment and fortification of cookies with dietary protein powder derived from green lentil that is rich in minerals and phytochemicals to partially meet the minimum nutritional requirements of kids. The proposed cookies may enhance the nutritional and industrial values of confectionary and bakery and their capacity may contribute to reduction in nutritional deficiency in children and to improve their nutrition outcomes.

Materials and Methods

Wheat flour and a premium Canadian green lentil were purchased from a local market in Kayseri city, Turkey. Green lentils were roasted on low heat in a frying pan and, after being cooled down, the seeds were milled until they became a fine flour in a commercial blender (MK-K45 Panasonic, Japan) (Figure 1).

Figure 1: *Green lentils and its fine powder and the final product.*

Manufacturing of cookies

Recipe and preparation of cookie without green lentil flour

A portion of 300g wheat flour was added to refined sugar (200g), corn starch (70g), skimmed milk powder (10g), vanilla (5g), baking powder (1g), and cinnamon fine powder (8g). After that, butter (125g), eggs (110g) and cheese whey (10 ml) were added and kneaded until they all became dough. Then the dough was rested at room temperature for 30 minutes. After shaping the dough into cookies, they are baked in an oven using consistent dry heat for 16 minutes at 180°C (Figure 2).

Figure 2: *Scheme shows the manufacturing outline of the proposed cookies.*

Recipe and preparation of cookie without green lentil flour (6 and 12%)

The same quantities of all ingredients except for the addition of green lentil powder, which came at the expense of the wheat flour quantity. The amount that was added from the lentils was subtracted from the portion size of the wheat flour. However, all the ingredients were added in the same amounts in the same preparation style as shown in the control cookies scheme (Table 1).

Ingredients in grams	Control	6%	12%
Wheat flour	300	250	200
Sugar	200	200	200
Green lentil powder	0	50	100
Eggs	110	110	110
Corn starch	70	70	70
Butter	125	125	125
Skimmed milk powder	10	10	10
Cheese whey	10	10	10
Cinnamon	8	8	8
Vanilla	5	5	5
Baking powder	1	1	1

Table 1: *Manufacturing formula and ingredients content of control cookie and cookies enriched with lentil flour at 6 and 12%.*

Protein analysis

Protein extraction

Proteins were extracted from the control and enriched samples by adding 28 ml of each extraction solution to 2g of the samples [15]. Proteins were extracted using three different solutions. Weber-Edsall buffer (0.6 M KCl) [16], while the second solution was a low-ionic-strength solution, which extracted proteins defined as water-soluble proteins (WSP) (50 mM imidazole-HCl pH 6.0, 2 mM EDTA). The third solution was a high-ionic-strength solution, phosphate buffer (0.09M KH_2PO_4, 0.06M K_2HPO_4, 0.3M KCl, pH 6.5) [17]. The solutions along with the samples were homogenized three times for 30s, at 10-s intervals, using a polytron homogenizer (Kinematica Co., Littau, Switzerland) at setting 4. While cookie samples were homogenized, we used ice to reduce any increase in the mixture temperature. The mixtures were then centrifuged at 4,000 rpm for 30 minutes at 4°C in a Himac CR 20E centrifuge (Hitachi, Tokyo, Japan). The supernatants were removed and filtered using filter paper No. 5A (Advantec Toyo K. Ltd., Tokyo, Japan), and the final solution was used as the extracted protein solution [18-20].

Proximate analysis and nutritional values

Each cookie sample was weighed in 4 parallel quantities at a fixed amount and then held at a stable temperature until 105°C, at which the amount of moisture was calculated. Ash is an inorganic residue remaining after the burning of organic materials. The inorganic part containing the minerals remains. Sodium, potassium, calcium, magnesium are present in excess. Iron, copper, zinc, manganese, aluminium, iodine, arsenic, fluorine and other trace elements are present in small quantities. The crates were dried in an ash oven. The crucibles were cooled down in the desiccator until the hard weighing was done. The crucible was weighed on four parallel pieces of each cookie sample. Samples were burned at 550°C until the colour of the samples turned white. After burning, the samples were cooled in a desiccator and the ash content of samples was calculated [21].

Kjeldahl method for determination of nitrogen

Nitrogen is one of the five major elements found in organic materials such as proteins. The Johan Kjeldahl method is widely used in determining the amount of protein in food samples. It is well known that the central basis used in this procedure is the oxidation of the organic compound using strong sulphuric acid. As the organic material is oxidized, the carbon is converted to carbon dioxide while hydrogen is converted into water. The nitrogen from the amine groups exists on the amino acid residues in food proteins, is converted to ammonium ion, which dissolves in the oxidizing solution, and can later be converted to ammonia gas. The sample is first denatured in a strong sulphuric acid in the presence of a catalyst, which helps in the conversion of the amine nitrogen to ammonium ions. The ammonium ions are then converted into ammonia gas, heated and distilled. The ammonia gas is led into a trapping solution where it dissolves and becomes an ammonium ion once again. Finally, the amount of the ammonia that has been trapped is determined by titration with a standard solution, and the final concentration is calculated [21-23]. Kjeldahl method: In the Kjeldahl procedure, after digestion in concentrated sulphuric acid, the total organic nitrogen is converted to ammonium sulphate. Ammonia is formed and distilled into boric acid solution under alkaline conditions. The borate anions formed are titrated with standardized hydrochloric acid, by which is calculated the content of nitrogen representing the amount of crude protein in the sample. Most proteins contain 16% of nitrogen, thus the conversion factor is 6.25 [21-23]. The point of inflection is between pH 4.4 and pH 4.7. If a titrator is used, one titrates for pH 4.65. A pH electrode is used with the titrator. It is always important to ensure that the electrode is in perfect condition and the instrument has been calibrated [22,24].

Protein concentration

Protein concentration (PC) was determined using the Biuret Method with biuret buffer as a standard. Each test tube contained 2.4 ml of biuret buffer and 0.6 ml of samples of K (control samples), A (6% green lentil flour) and D (12% green lentil flour). In addition, 2 blind and 3 replicates were prepared. Then samples were vortexed and waited in a dark place for 30 minutes. Absorbance of samples was measured with a spectrophotometer (UV-1800, UV spectrophotometer, SHIMADZU) at 540 nm. And then protein concentrations were calculated according to the following formula [25,26]: Protein Concentration (mg/ml) = (abs of sample – abs of blind)*20.

Determination of changes in protein molecular weights

Proteins extracted from samples were separated into gel sheets of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to determine any changes in their molecular weight (MW). Samples were dyed with β-mercapto ethanol-bromophenol blue and loaded onto gradient gel made of 7.5% (to visualize high-MW proteins), and 20% (to visualize all proteins) [27]. Electrophoresis was carried out at a constant current of 30 mA/gel with a Mini protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After electrophoresis, gels were stained with 0.25% (w/v) Coomassie Brilliant Blue R-250 for 15h and then distained with 50% (v/v) methanol solution for 4 hours. The patterns with the clearest separation were photographed [20].

Colour measurements

Colour measurement was carried out using a colorimeter and the values were expressed as L* for lightness; a* for redness vs greenness and b* for yellowness vs blueness respectively (Konika Minolta Chromameter CR-5) [18]. Measurements (n=3x6 replicates) were taken for all the samples. The colour analysis was directly performed on cookies at different spots.

Sensory analysis

Principally, organoleptic properties' analysis was designated to evaluate the panel's preference and level of palatability towards those cookies made without or with green lentil powder. Normally, sensitivity to flavouring compounds is made by the taste buds that are located on small bumps on the tongue called fungiform papillae. Those fungiform contain a huge number of receptors, and their numbers differ from one person to another that bind to different small molecules related to flavour and aroma. Each type of cookie was identified with a code that the panellists were not aware of its composition. A questionnaire for the evaluation was prepared according to ISO 16820:2004 and ISO 6658:2005 [28]. To recalibrate the taste sensation information for the brain, fresh water was provided to rinse the mouths of the panellists along with the samples. There were 50 panellists (40 women and 10 men), aged from 21 to 44 years old who participated in the study, who accepted voluntarily to do the evaluation and to be involved in the study. Forty panellists who participated in the sensory evaluation were semi-trained, while the rest of 10 were professional and were well-experienced. The panellists evaluated the baked cookies for total taste (intensity of taste), smell, sweetness, colour, appearance, breakability, swallowing ability, chewiness and size. Participants had to score the acceptability using a five-point hedonic scale ranging from 1 ("dislike very much") to 5 ("like very much"). The panellists were allowed to sense and record responses concerning the intensity of sensory attributes of the three types. The data is a collection of the positive responses against each attribute that is subtracted from the total panellists' responses and expressed as means of percentage.

Statistical analyses

Statistically the results were evaluated at SigmaPlot 11.0 statistics package program. Tukey multiple comparison test was used to determine differences between groups by applying single factor analysis of variance (ANOVA).

Results and Discussion

Proximate analyses and nutritional values

The moisture content of the cookie without green lentil flour was 85%. On the other hand, a cookie with 6% lentil flour had more moisture content (90.2%) than the cookie without lentil. Furthermore, a cookie with 12% green lentil flour had the greatest moisture content (91.4%) among all samples (Figure 3). This result shows that the green lentil flour absorbs water more than the others and helps to increase the chewiness of the cookies. The findings of moisture in 12% of lentil samples are in agreement with the chewiness data, which was the highest among other samples. Generally, the addition of 6 and 12% lentils to the baseline cookie has contributed to increasing the moisture by 5.2 and 6.6%, respectively.

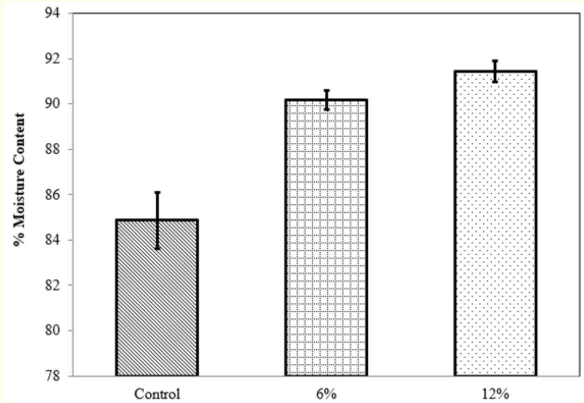

Figure 3: *Moisture content (%) of cookie samples as affected by lentil addition (± SDV).*

Data showed that the cookie without lentil flour had 0.70% ash content (Figure 4). Despite being the cookie with 6% lentil flour, it has more ash content than without lentil (1.11%). Furthermore, the cookies with 12% lentils were shown to have even greater ash content, 1.224%. Data suggests that cookies with higher lentil flour show higher ash content in comparison to other samples. The reason is that as the green lentil ratio increases (6 and 12%), the mineral content increases by 61.8 and 77.5% on the baseline value (Figure 4). These results are in accordance with data of the moisture content and protein content. From the nutritional point of view, the addition of lentils is quite valuable in terms of protein content and ash percentage. It is well understood that food rich in ash highly contributes to kids' health and bone as well as muscle development. Generally, childhood obesity, if not given serious attention, will lead to various health risks as children mature, such as hypertension, heart disease, gout, cancer, diabetes, or osteoporosis [29]. Osteoporosis is a systemic skeletal disease characterized by low bone density and caused by micro-architectural deterioration giving rise to bone fragility and an increased susceptibility to fracture. Osteoporosis is an important health problem occurring both in developed and developing countries [30]. However, such cookies may play a crucial role in kids' health that tackles common diseases including osteoporosis if offered in proper quantities.

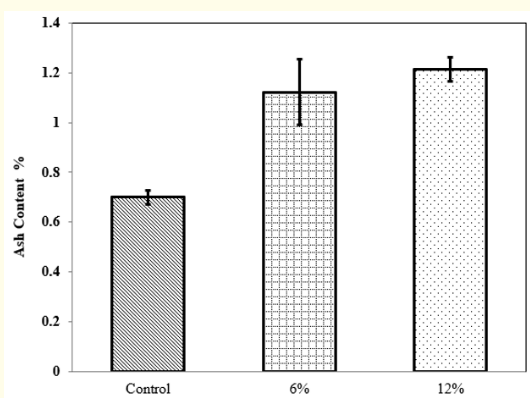

Figure 4: *Ash content (%) of cookie samples as affected by lentil addition (± SDV).*

According to protein results, samples of 6% of lentils had the greatest protein content (8.14%) compared to the other samples. In contrast, the control sample had the lowest protein content (6.8%) (Figure 5). It is clear that the addition of green lentil increased the protein content by 20%, as lentil contains more protein than in wheat flour. Surprisingly, 12% of lentil cookies have a lower protein content than A sample. Luckily, lentil is low in some fats, generally lentil has a low concentration of lipids, ranging from 0.3 - 3.5 g/100g dry matter [31]. Generally, the 12% addition of lentils did not contribute to the increase in protein content, which might refer to the extraction ability.

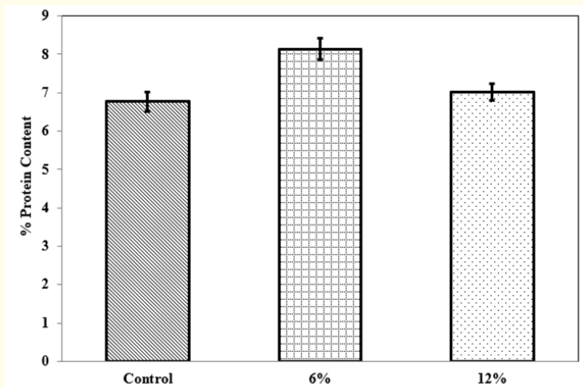

Figure 5: *Protein content (%) of cookie samples as affected by lentil addition (± SDV).*

Protein solubility and concentration

Cookie samples enriched with 6 and 12% green lentil flour showed the highest extraction ability of all buffer types. Despite the fact that the control samples extracted in Weber-Edsall (WE), water low ionic buffer (WSP), and phosphate buffer (PB) have lower protein extractability than the fortified samples. Surprisingly, solubility of control samples that were extracted by WSP buffer had the lowest value. The WSP buffer usually extracts proteins that hydrophilic and mitochondrial enzymes, therefore the ratio is always low. The results suggest that the samples of cookies enriched with 6 and 12% lentils had higher protein extractability, which is highly related to the addition of lentil (Figure 6). The increase of protein solubility in the WE, SWP and phosphate buffers was statistically significant when compared to their counterparts in the control samples. However, the values of protein solubility in the 6 and 12% enriched-lentil cookies were insignificantly different. Plainly, the increase in protein solubility in the fortified samples has altered the nutritional values, although, Ahhmed and others stated in previous reports suggests that if solubility of protein increases the stability decreased [20]. Obviously, the results indicated that there was a great corresponding correlation between high lentil concentration and protein extractability and solubility. In summary, the addition of lentils has increased protein content and protein solubility, which in turn may increase the digestibility of proteins in cookies. The data may contribute to the value of lentils as precious ingredients that could be utilized in functional foods for kids.

Results of changes in protein molecular weights (SDS-PAGE)

The control samples extracted in Weber-Edsall (WE) and phosphate buffer (BP) exhibited proteins with molecular weights that ranged from 14 - 60 kDa. Although there is a difference in protein band intensity, still the number of proteins existing in both buffers is almost the same. In control samples, the phosphate buffer-PB is the stronger in terms of extracting all types of protein. However, data also

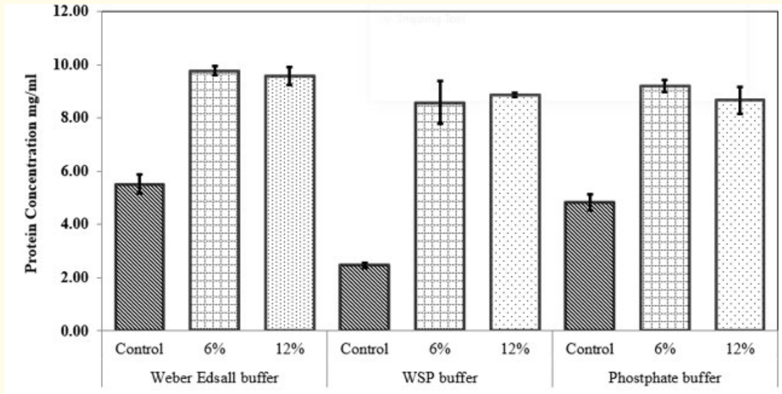

Figure 6: *Protein concentration of cookie samples as affected by lentil addition (mg/ml) (± SDV).*

suggest that K3 (water soluble protein-WSP) was the weakest buffer in extracting proteins of all ingredients. This buffer could extract only lysozyme, α-laktoalbumin (14 kDa), β-casein (24 kDa), α, β,γ-gliadins (37 kDa) and finally, IgG (45 kDa) (Figure 7). WSP seemed to have very dissimilar extraction ability of protein ingredients to the ability of WE and BP. Data clearly suggest that major proteins from the main ingredients (wheat, egg, whey and milk proteins) coexisted in the filtrates extracted in those buffers, although differences were observed.

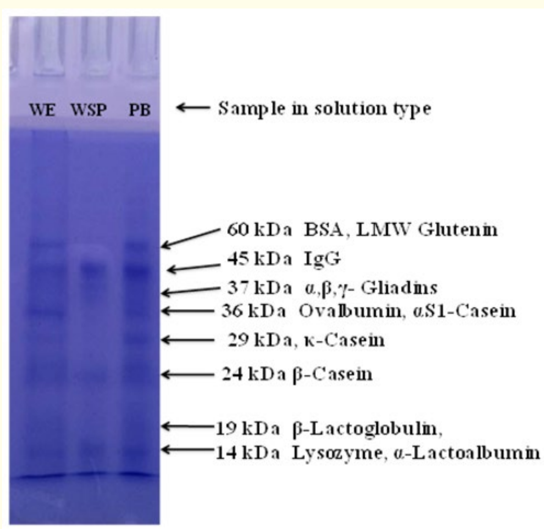

Figure 7: *SDS-PAGE pattern shows MW changes of major proteins extracted by different buffers from control cookie as affected by processing. Weber-Edsall buffer (WE); Imidazole buffer (WSP) and phosphate buffer (BP).*

Data from figure 8 suggests that samples of 6% addition and extracted in WE and PB showed quite a similar and stronger extraction ability to all proteins found in all ingredients. WE and BP could extract lentil proteins covicillin (66 kDa), vacillin (60 kDa), legumin IIS acidic subunit (50 kDa) that were not detectable in control samples. Furthermore, they can also extract lectin (29 kDa), legumin IIS basic subunit (25 kDa) and finally γ-vicilin (19 kDa) (Figure 8). This is strong evidence that the addition of lentils could obviously have contributed to the enrichment of cookies with new biological compounds that can offer bioactive functions. Usually, proteins from lentil offer functional proteins and bioactive peptides that can be utilized in food processing, such as utilized cookies and other confectionary products. Moreover, those WE and phosphate buffers could extract proteins from skimmed milk powder, whey and egg proteins such as BSA (60 kDa), IgG (45 kDa), ovalbumin, αSI-casein (36 kDa), κ-casein (29 kDa), β-casein (24 kDa) and β-lactoglobulin (19 kDa) and eventually lysozyme and α-lactoalbumin (14 kDa). Surprisingly, the expression of glutenin proteins (60 kDa) and α, β,γ-Gliadins (37 kDa) was not quite high in filtrates of WSP buffer, which may be due to low ionic strength and their extractability might be weak in water. Figure 8 also indicates that the WSP buffer of 6% sample was not able to extract three major protein groups including covicillin (66 kDa), BSA, vacillin, LMW glutenin (60 kDa) and ovalbumin and αSI-casein (36 kDa). Clearly, data suggest that expression of lentil proteins was to a great extent, which was not available in the case of data in figure 7. Again from SDS-PAGE pattern, the article suggests that the nutritional value was increased in cookies after being fortified with lentils. Data suggest that egg and whey proteins were also a source of nutritional components including bioactive peptides. Whey proteins is a source of α-lactalbumin, β-lactoglobulin, bovine serum albumin, caseinomacropeptides, immunoglobulins, lactoferrin, lysozyme which are often associated with health benefits, such as enhanced immunity, anticancer properties, and adhesive effect against pathogenic properties, as well as antiviral, antimicrobial (iron binding properties) and antihypertensive properties [32].

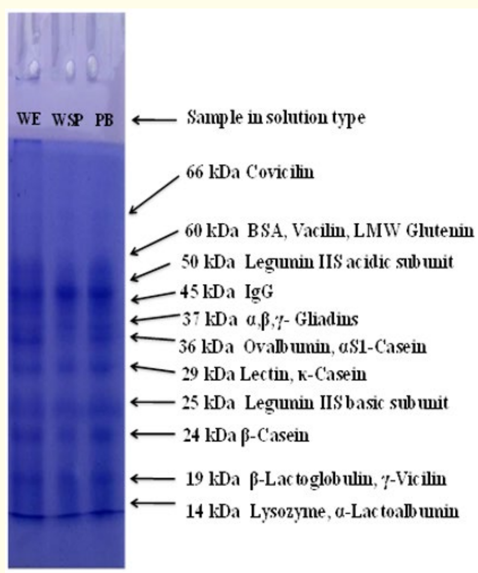

Figure 8: *SDS-PAGE pattern shows MW changes of major proteins extracted by different buffers from cookie enriched with 6% lentil flour as affected by processing. Weber-Edsall buffer (WE); Imidazole buffer (WSP) and phosphate buffer (BP).*

Data from figure 9 illustrates that samples of 12% of the lentils, which were extracted in WE, WSP and PB indicated similar extraction ability to all proteins that exist in the major ingredients. The three buffers could extract lentil proteins covicillin (66 kDa), vacillin (60 kDa), legumin IIS acidic subunit (50 kDa). Additionally, they also could extract lectin (29 kDa), legumin IIS basic subunit (25 kDa) and γ-vicilin (19 kDa). Furthermore, all those buffers could extract the same proteins detected in the control samples, such as low molecular weight proteins, glutenin proteins (60kDa) and α, β and γ Gliadins (37 kDa) are shown in figure 6. Ironically, WSP buffer showed the same force of other buffers in extracting the major proteins. The reason is not understood yet, but from the nutritional point of view, the current data would enhance the value of adding lentils to kids› food (Figure 9). From the images of the SDS-PAGE obtained in this research, a conclusion can be said that the nutritional value of the novel cookie is quite high due to the mixture of protein type, size and biological quality. The results, may inspire food manufacturers to include nutritional ingredients that are neglected and absent from appreciation in the baby food industry. The addition of such ingredients as lentils would strengthen kids' food from the nutritional aspects and that would result in reduction of high-glycaemic ingredients such as wheat and barley flour.

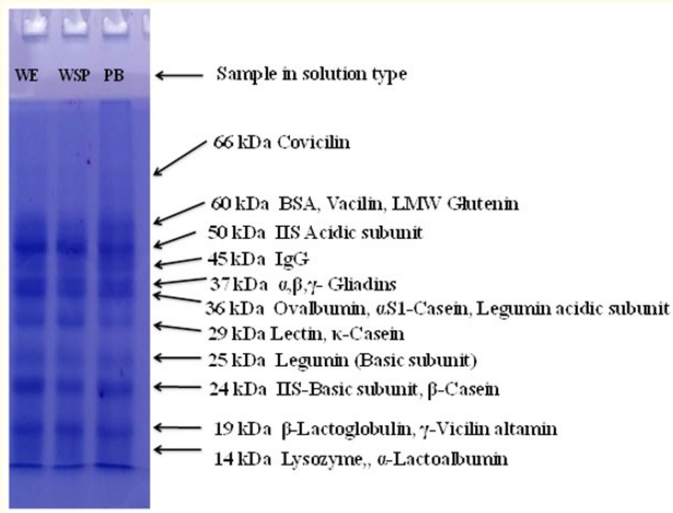

Figure 9: *SDS-PAGE pattern shows MW changes of major proteins extracted by different buffers from cookie enriched with 12% lentil flour as affected by processing. Weber-Edsall buffer (WE); Imidazole buffer (WSP) and phosphate buffer (BP).*

Colour values

The surface colour of cookies and confectionaries together with their texture and taste are very important elements for the initial acceptability by consumers. The whiteness index mathematically combines lightness and yellow-blue into a single term and has been used to compare colour intensity. Figure 10 shows differences in colour values (L^*) of the control, 6 and 12% enriched lentil flour samples. L^* value of the control sample has high lightness that is insignificantly higher than 6% enriched cookies but significantly higher than 12% enriched cookies. It is clear that the rate of decrease in whiteness colour intensity is strongly due to the effect of lentil addition, simply because its flour colour is greenish. The gradual decrease in the density of white is accompanied by a gradual increase in the proportion of added lentils. The higher the percentage of lentils, the lower the white density, which clearly indicates that there was a directly inversed relationship. Values of a^* are statistically different among all the samples (figure 11). Results illustrated that samples enriched with 6% lentils had the highest a^* values, while control samples had the lowest value. Cookies fortified with 12% lentil showed a greater a^* value that was significantly higher than the control and 6% enriched samples. The gradual increase in the density of the redness (dark) is accompanied by a gradual increase in the proportion of added lentils. The higher the percentage of lentils, the higher the red density, which clearly indicates the relationship was directly correlated. Perhaps the addition of lentil contributed to Millard reaction during baking, which results in a higher a^* value. The addition of lentils may also contribute to the increase of acceptability of consumers as of the brownish baking colour.

Analytically, the b^* value is an indicator of yellowish vs blueness colour. As a result, A sample statistically has the highest b^* value among all samples, due to the wheat and green lentil component. Despite that, the 12% sample had the lowest value because (Figure 12). The difference between b^* values in control and 12% enriched cookies is insignificant. Yet, the difference between b^* values in 6 and

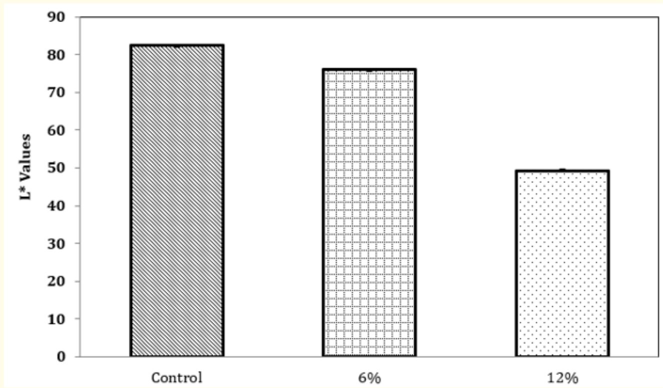

Figure 10: *L* value of cookie samples as affected by lentil addition.*

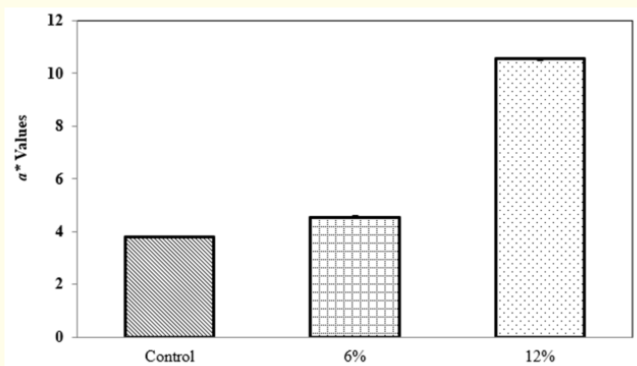

Figure 11: *a* value of cookie samples as affected by lentil addition.*

12% enriched cookies is significant. The unsystematic results of *b** are not well understood, but it is clear that the higher addition of lentil contributed to a higher blueness value and lower yellowness score. Therefore, a 12% lentil addition might be a secondary option as it may reduce acceptability of consumers for the product, since the beige or light brown colour of cookies is preferred by consumers.

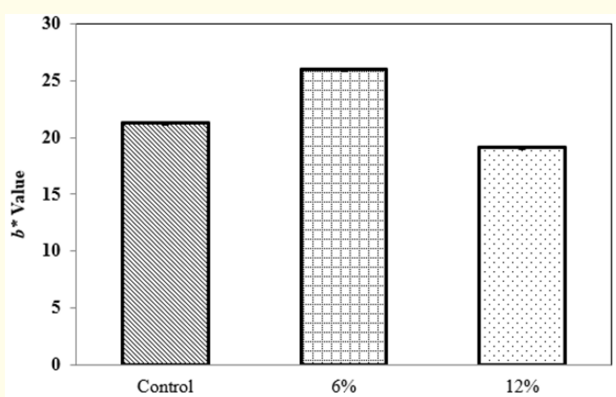

Figure 12: *b* values of cookie samples as affected by lentil addition.*

Evaluation of sensory properties

Sensory evaluation of cookie without green lentil

Smell and taste are the most important parameters in preference to foods. Participants voted 27.5% for "bad", although they voted 32% "quite good" for the smell of the control samples (Figure 13). On the other hand, participants voted 65.5% "quite good to good" for the taste of the K sample. When compared to the other samples, the smell of the K sample has the lowest vote. It is also possible that the lowest vote is due to the fact that wheat flour and other components are not homogeneously dispersed in cookie manufacturing, and it may be due to the familiarity of general cookies.

As shown in figure 13, participants voted 37.5% "good" for the colour of the control samples. While 30% of the votes in the assessment were "bad". This sensory value is the lowest compared to the other samples. Because the control sample was made of wheat flour, which contributed to the whiteness that was lighter than the others. On the other hand, participants were voted 35% "bad", even though the appearance of the K sample had 50% votes as "good". This rate is related to a sample of the outer homogeneous colour, size and shape. Data shows that participants voted 55% for "bad", although they voted 42.5% as "good" for breakability of the control sample. When compared to the other samples, breakability of the control sample has the lowest vote. This may be due to the fact that the moisture content of the sample and the components are homogeneous. On the other hand, participants voted 30% as "good" and 32.5% as bad for the swallowing ability of the control sample. The high rate of negative assessment might be due to the mowing property of cookies that made panellists feel like cookie stucks in the throat, which increases drooling and sometimes refluxing the cookie after swallowing, known as regurgitation. Participants also voted 27.5% as "good" and 30% as "bad" for chewiness of the baseline samples. Chewiness was also low due to low breakability and wheat flour content. On the other hand, participants voted 30% as "good" and 17.5% as "bad" for the size of the control sample.

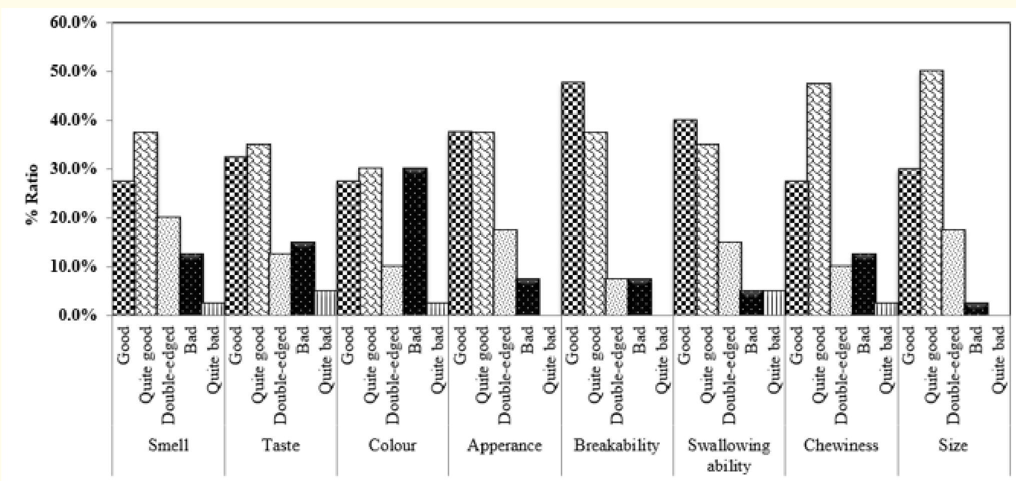

Figure 13: *Sensory evaluation of cookies enriched with green dietary lentil flour (12%), estimated by 50 trained panellists.*

Sensory evaluation of cookie with 6% green lentil

The descriptive and hedonic sensory data show that participants voted 82.5% as the sum of "good and quite good" for smell of the 6% enriched sample (Figure 14). This rate is higher than the other samples, obviously. In addition, feedback on the taste of 6% lentil added samples was higher than the others. It seemed that the lentil after being baked with a cookie contributed to a better smell and taste (flavour). The result shows that panellists input 70% of the sum of "good and quite good" for colour of the 6% enriched samples. On the other hand, A sample has the same value of votes for colour as for appearance (72.5%). Samples enriched with 6% lentils illustrated the highest colour rate compared to other samples, which is very positive feedback that contributes to the advantages of the novel cookies made with lentils.

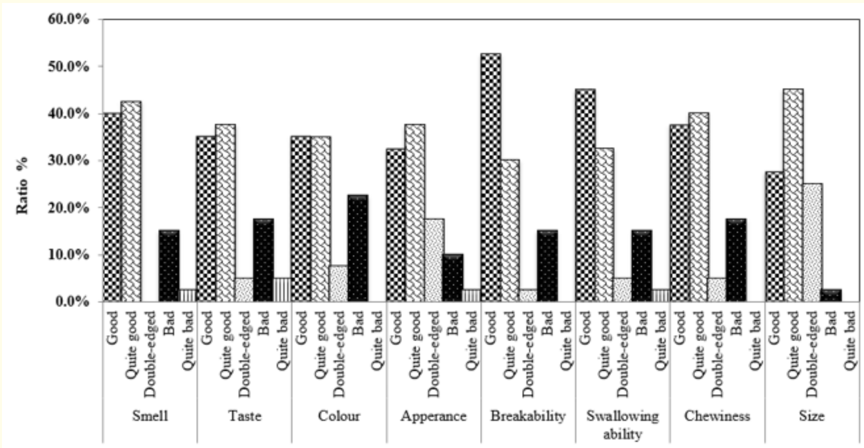

Figure 14: *Sensory evaluation of cookies enriched with green dietary lentil flour (6%), estimated by 50 trained panellists.*

Estimates of participants were 82.5% as "good and quite good" for breakability, which is the highest compared to the other samples. On the other hand, the same sample received the highest score of swallowing ability (77.5% sum of good and quite good). Participants' inputs on the sum of "good and quite good" for the chewiness and swallowing ability of the 6% enriched sample were 77.5 and 67.5%, respectively. Additionally, participants' feedback was 72.5 and 72.5% as "good and quite good" for the parameters of chewiness and size of this sample.

Sensory evaluation of cookie with 12% green lentil

As shown in figure 15, participants voted 77.5% as "good and quite good" for both smell and taste of the 12% enriched sample. This sample has the highest taste score, which might be due to lentil or the perfection of the combination. As shown in figure 15, feedback of participants was 67.5% as "good and quite good" for the colour, while 77.5% for the appearance, which was the highest rate. Panellists gave a score of 70% as "good and quite good" for the breakability, while its rate of swallowing ability was 82.5%, the highest among the samples. Data suggest that the rate of chewiness and swallowing ability were 82.5 and 77.5% for the 12% enriched samples. In summary, food innovation may allow better palatability, which would eventually lead kids to increase the functional food intake. A positive parental role model may be a better method for improving a child's diet than attempts at dietary control [33]. As a matter, increasing the utilization of fresh fruits, vegetables, food products containing natural additives such as vitamins, minerals, fiber and natural antioxidants to overcome the problems of malnutrition [34].

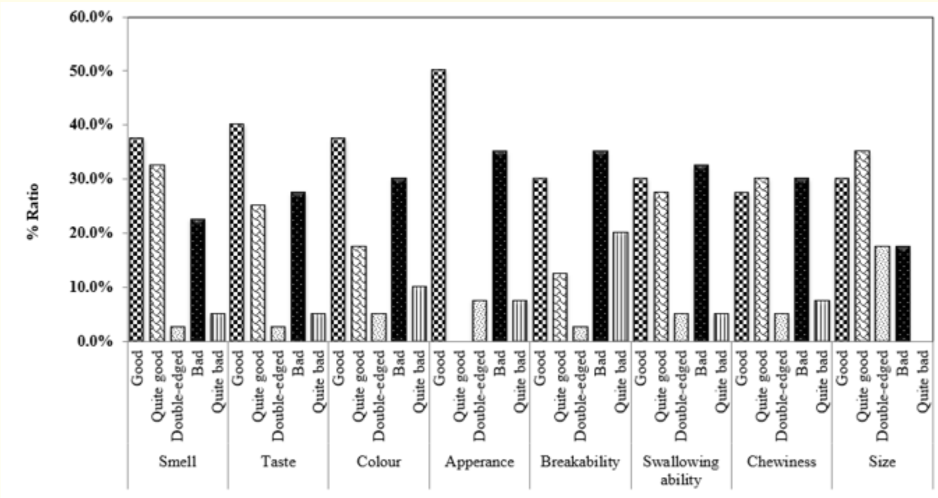

Figure 15: *Sensory evaluation of the control cookies estimated by 50 trained panellists.*

Conclusion

According to protein results, the enriched sample with 6% lentil had the most protein content, 9.35%. Meanwhile, the control cookie samples showed the lowest protein content. According to the results of the analysis, the samples containing green lentil showed the highest extractability by all buffer types. Surprisingly, protein extractability WSP buffer from the control sample was the lowest. Data suggest that the higher the percentage of lentil addition, the higher the protein content and solubility, which clearly indicates that nutritional values directly correlated with the addition of lentil. As a result, dry matter and ash exhibited high rates in the cookies fortified with green lentil flour. Phosphate buffer is a strong in terms of extracting all types of protein of the major ingredients. Fortified samples (6 and 12%) exhibited the major lentil proteins including covicillin (66 kDa), vacillin (60 kDa), legumin IIS acidic subunit (50 kD), lectin (29 kDa), legumin IIS basic subunit (25 kDa), and γ-vicilin (19 kDa). Adding green lentil dietary flour could also contribute to the consumer acceptability of the fortified cookies because the colour of the samples has been improved, despite the fact of the fluctuation in L^*, a^* and b^* values. Generally, sensory analysis data showed that the 12% enriched sample had the highest rating, while the control sample received the lowest rates. To the best of our knowledge, determination of the nutritional and functional properties of cookies enriched with green lentil dietary flour proposed for kids is the first of its aim. In summary, cookies fortified with green lentil dietary flour is of great importance and considered as healthy and functional children's food since the nutritional values of the enriched product have been observed. The product became rich in protein, probiotic by-products, phytochemicals, dietary fibre, and extra added minerals. Thus, the utilization of green lentils dietary flour may remarkably reduce the challenge of nutrient deficiency in kids.

Conflict of Interest

The authors declare that the research was conducted with no specific funding, nor conflict of interest.

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