Retention of bioactive compounds during extrusion processing and storage

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ABSTRACT

Retention of bioactive compounds (beta-glucans, lignans and gamma oryzanol) was analyzed after extrusion and during storage period of three months under four relative humidities using saturated salt solutions such as potassium carbonate (43.26%), magnesium nitrate (52.60%), potassium chloride (84.36%) and potassium nitrate (93.58%). The control sample comprising a corn and rice flour blend (50 g each) was substituted with beta-glucans at 3 g/100 g and 6 g/100 g, flaxseed lignans at 6.67 g/kg and 11.67 g/kg and gamma oryzanol at 1.5 g/100 g and 3.0 g/100 g at low and high levels, respectively. After extrusion, beta-glucan was retained up to 82.67 and 90.83%, lignans were retained at 86.31 and 66.66% whereas retention of gamma oryzanol was 71.33 and 51.67% at low and high level of substitution, respectively. Retention of bioactive compounds was the lowest along with a decrease in $L*$ and $b*$ values and an increase in $a*$ value was observed under higher relative humidity (84.36% and 93.58%) storage conditions.

Introduction

Owing to an increasing awareness of consumers towards health, there is an upsurge in the development of functional foods. In the previous three decades, the importance of the role of dietary fibre in maintaining the overall health of human beings has been able to attract significant scientific interest (Brennan & Cleary, 2007). Beta-glucan is a type of soluble fibre which forms a viscous solution in the digestive system (Choo & Aziz, 2010). These are polymeric forms of d-glucose molecules comprising β-glycosidic bonds having widespread distribution in cereals (mainly oats, barley), bacteria, yeast, fungi (including mushrooms) and seaweeds (Zhu et al., 2015). Rahar, Swami, Nagpal, Nagpal, and Singh (2011) reported that in yeasts and mushrooms, β-glucan contains (1 → 3) linkages with (1 → 6) linkages, whereas cereal grains such as oats as well as barley comprise of (1 → 3) and (1 → 4)

linkages. Over the years, beta-glucans have been able to achieve huge attention owing to numerous health benefits recognized by EFSA (EFSA, 2011). As per the recommendation made by FDA, significant health benefits can be met by consumption of 3 g of beta-glucan per day or foods containing 0.75 g of beta-glucan per serving on daily basis (Thondre et al., 2011). One of the best ways to incorporate dietary fiber fractions into the structure of the final product is the extrusion technology (Alam, 2012). There is an abundant literature which reports about the utilization of beta-glucans primarily as dietary fibre in food products based on cereals such as biscuits as well as cookies (Brennan & Samyue, 2004), bread (Brennan & Cleary, 2005) and cakes (Lee et al., 2004). Brennan and Cleary (2007) utilized Glucagel, a β-glucan isolate from barley (*Hordeum vulgare*) to enhance the nutritional profile of bread by incorporating it at 2.5% and 5%. Choo and Aziz (2010) incorporated the oat beta-glucan and mature green banana flour into noodles to

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analyze their nutritional and sensory characteristics. Hence, one of such an attempts has been made in this study in the development of beta-glucan enriched extrudates by substituting blend of corn-rice flour with isolated beta-glucan concentrate at low (3 g/100 g) and high (6 g/100 g) level.

One of the functional food sources that is being widely used is flaxseed due to its unique nutritional profile and potential in reducing the risk of cardiovascular disease and hormone dependent cancers such as prostate and breast. (Adlercreutz et al., 1992). One of the growing reasons behind the popularity of flaxseed is due to its significantly high content of lignans, mainly secoisolariciresinol diglucoside (SDG) (Alhassane & Xu, 2010). These compounds have been acknowledged in curbing hormone-related cancers owing to the similar structures of enterolignans and mammalian oestrogens (Sainvitu et al., 2012). One of most prominent flaxseed lignans, SDG holds its relevance commercially in the world market but its utilization in food needs to be introduced. Muir and Westcott (2000) conducted a work on the incorporation of flaxseed meal, aqueous alcoholic extracts of flax seed and pure SDG (purity 82%) into a white bread mix. Macaroni was fortified with whole ground flaxseed at different levels (10%–20%) followed by drying at 40 °C, 70 °C and 90 °C. Significant recovery of SDG was observed and it was also found stable during the storage period (Hall et al., 2005). Stability of SDG was also reported in various bakery products during storage at ambient temperature and deep-frozen form (Hyvärinen et al., 2006a).

Over the years, the interest in the formulation of novel and functional food products supplemented with antioxidants derived natural origin in the form of fruits, vegetables, whole grains and oilseeds. Out of several natural sources, rice bran contains adequate level of several phytochemicals which possess antioxidant activities apart from other health promoting properties (Chen & Bergman, 2005). Gamma-oryzanol has secured potential significance as a naturally derived antioxidant from rice-bran oil stabilizing food and pharmaceutical products (Sapino et al., 2013). It is primarily a polyphenolic compound which resembles another component of rice bran oil, tocopherol (Godber et al., 2002). Gamma oryzanol has gained immense significance as a food and medical antioxidant in Japan which is one of the strongest contenders in bioactive world (Suh, Yoo, & Lee, 2007).

Kumar, Sai Manohar, Indiramma, and Gopala Krishna (2014) analyzed the variations in the physical characteristics and sensory characteristics of biscuits fortified with oryzanol concentrate and control biscuits. The oryzanol incorporated fat (2.4% oryzanol) was utilized for the preparation of oryzanol fortified biscuits. An insignificant variation was observed in fat content of control biscuits and oryzanol fortified biscuits during storage period at varying relative humidities-11, 22, 32, 44 and 56%. Ohtsubo et al. (2005) processed pre-germinated brown rice using a twin screw screw extruder and analyzed oryzanol, inositol, total ferulic acid and total dietary fibers in extruded pre-germinated brown rice. Oryzanol content of unextruded polished rice came out to be 6.1 mg/100 g where as it was 50.4 mg/100 g and 19.7 mg/100 g for germinated brown rice and extruded germinated brown rice, respectively.

The present study was conducted to analyze the retention of beta-glucans, flaxseed lignans and gamma oryzanol in products after extrusion as well as during storage period of three months in which the optimized extruded samples were evaluated at time interval of 15 days for moisture content, textural hardness, color and retention of bioactive compounds with respect to beta-glucan content, lignan (SDG) content and gamma-oryzanol content.

Material and methods

Raw materials

Corn and rice flours were obtained from Radhey rice mills from Mahindra Rice Mills, (Dhuri) district Sangrur (Punjab) India. Beta-glucan concentrate (β-glucan: 65.74 g/100 g, w.b.) was isolated from barley flour by using the methodology of Kour, Singh, and Saxena (2018). Flaxseed lignans with secoisolariciresinol diglucoside (SDG) content (30 mg/g) was extracted from defatted flaxseed powder by following the methodology of Kour et al. (2018). Gamma oryzanol was extracted from rice bran oil by following the methodology of Kour et al. (2018). The bioactive compounds extracted were stored at 4 °C till further analysis.

Chemicals and reagents

Beta-glucan estimation kit was procured from Megazyme International Limited (Megazyme International Ireland Ltd., Wicklow, Ireland). The standard of Secoisolariciresinol diglucoside having purity ≥ 97 % was purchased from Sigma Aldrich, (St. Louis) (Missouri, USA) whereas gamma-oryzanol standards were procured from Tokyo Chemical Industry, Chuo city, Tokyo, Japan.

Extrusion

Extrusion of corn-rice flour mixture fortified with low and high level of beta-glucan concentrate, flaxseed lignan concentrate and gamma oryzanol concentrate was carried out by conducting the experiments under the predicted values of optimized processing conditions obtained by numerical optimization technique by using a co-rotating twin-screw extruder (M/s. Basic-Technology Pvt. Ltd. Kolkata, India). The independent variables were barrel temperature (90–110 °C), moisture content (17–21%), and screw speed (270–290 rpm). Response surface methodology (RSM) which is a major tool in analyzing experimental data was employed to derive the optimum levels of the independent variables by using a three factor, five level central composite rotatable design (CCRD). The dependent variables or responses selected were radial expansion, bulk density, water absorption index, textural hardness, sensory score and colorimetric values ($L*$, $a*$ and $b*$). Optimization was carried out to develop a product with maximum radial expansion, sensory score, $L*$ value, $b*$ value and minimum bulk density, water absorption index, textural hardness and $a*$ value of fortified extrudates at both levels using numerical optimization technique.

At low level fortification with beta-glucans, the optimized conditions were barrel temperature (101.34 °C), screw speed (277.99 rpm) and feed moisture (17.00 %). At high level fortification with beta-glucans, the optimum conditions were barrel temperature (101.73 °C), screw speed (280.85 rpm) and feed moisture (17.00 %). The optimized conditions at low level fortification with lignans were barrel temperature (102.45 °C), screw speed (270 rpm) and feed moisture (17.00 %). For high level fortification with lignans, the optimized processing conditions were barrel temperature (108.65 °C), screw speed (286.17 rpm) and feed moisture (17.00 %). For low level fortification with gamma oryzanol, the optimized conditions were barrel temperature (110 °C), screw speed (285.387 rpm), feed moisture (17.00 %). For high level, the optimised processing conditions were barrel temperature (105.46 °C), screw speed (283.57 rpm), feed moisture (17.00 %). The product after coming out of die was cut with a sharp edged rotating knife and then the extrudates were dried at 60 °C for 7 h to remove undesirable moisture. The extrudates were stored in high density polyethylene (HDPE) bags away from heat and light till further analyses.

Moisture content

Moisture content of optimized extruded samples fortified with beta-glucan, flaxseed lignans and gamma oryzanol concentrate was determined by AOAC method 930.15 (2000). 2 g milled sample was weighed into an aluminium dish with a cover followed by drying in an oven at 135 ± 2 °C for 2 h. The dish with its cover was then transferred to a desiccator and then cooled. The weight of dried sample was measured till the constant reading. Loss of moisture was measured on final drying.

$$Moisture\, content (\%) = \frac{W_1 - W_2}{W_1} \times 100$$

Where, W_1 = mass of sample before drying (g); W_2 = mass of sample after drying (g).

Textural hardness

The textural hardness of the optimized fortified extrudates was determined by using a TA-XT2 Texture Analyser (Stable Micro systems Ltd., Godalming, UK). Each extrudate (6 cm long) was compressed with a target mode distance of 3 mm. A three point bending rig with a pre-test speed of 1.00 mm/*sec*, test speed of 2 mm/*sec* and post-test speed of 10 mm/*sec* was used. Load cell of 50 kg was used. Mode distance was kept at 3 mm. Each sample was analyzed in triplicates. A curve was generated with the force over distance due to compression.

Colorimetric values (L*, a* and b*)

The color of the optimized fortified extrudates was estimated by using a hand held colorimeter (CR-400, Konica Minolta, Chroma meter, Osaka, Japan) based on *L**, *a** and *b** values. *L** value denotes the lightness or brightness varying from 0 to 100, *a** value relates to the redness or greenness, with an increase in positive *a** value denoting more redness whereas the *b** value relates to yellow and blue color with an increase in positive *b** value signifying higher yellowness of the product.

Analysis of retention of bioactive compounds in the optimized samples after extrusion and storage

Beta-glucan estimation in optimized sample

Beta-glucan content in optimized fortified extrudates was determined by following the methodology of McCleary and Codd (1991) with slight modifications using megazyme mixed linkage beta-glucan estimation kit (Megazyme International Ireland Ltd., Wicklow, Ireland). 200 mg of sample was added to a glass centrifuge tube. Aqueous ethanol (5.0 mL, 50 % v/v) was added to the tubes followed by incubation in a hot water bath for about 5 min and vortexing the mixture. Ethanol (5 mL, 50 % v/v) was further added and the mixture was centrifuged at 3000 × g for 10 min. The pellet obtained was re-suspended in ethanol (5 mL, 50 % v/v) with constant vortexing. It was followed by the addition of 20 mM sodium phosphate buffer (4.0 mL) (pH 6.5) with vigorous stirring.

Incubation of the mixture was done in a boiling water bath for 5 min at 50 °C. followed by the addition of lichenase (0.2 mL, 10 U) to the tubes. Incubation of sealed tubes was done at 50 °C for 1 hr along with constant vortexing. Centrifugation was done for 10 min at 1000 × g. 0.1 mL of aliquots were dispensed into the bottom of the test tubes. Addition of β-glucosidase (0.1 mL) in 50 mM acetate buffer was done with incubation at 50 °C for 10 min. 3.0 mL of the glucose-oxidase–peroxidase reagent was added prior to incubation at 50 °C for 25 min. Test tubes were taken out from the water bath and measurement of absorbance was taken against blank at 510 nm.

$$\beta - glucan(\%w/w) = \Delta E \times F \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

Where, Δ_E is the absorbance taken after treatment with β-glucosidase minus blank absorbance, F is a factor depicting conversion of absorbance values to μg glucose = [100 (ug of glucose)/absorbance for 100 μg of glucose], FV relates to final volume (6.4 mL for extruded cereal products), 1/1000 determines conversion from micrograms to milligrams, 100/W is factor which determines β-glucan content in terms of percentage of sample weight, W (mg) is the dry weight of the sample analysed, 162/180 is a factor which converts free glucose to anhydro-D-glucose present in β-glucan.

Chromatographic analysis of lignan (SDG) in optimized sample

In the optimised samples having lignan fortification, SDG analysis was done to determine the retention of lignans by using the method of Li et al. (2008). The active ingredient (SDG) was identified and quantified by using an analytical HPLC instrument (HPLC Agilent 1260 series, Santa Clara, California, United States). Weighing of the samples as well as the standards was done using a Mettler-Toledo XS204 Excellence XS analytical balance (Polaris Parkway, Columbus, USA) having sensitivity of ± 0.1 mg. Sample was prepared by using a rotary evaporator (Recirculating Chiller F-305, Flawil, Switzerland). The equipment used had G1311B/C quaternary pump, G1329B autosampler, G1316B thermostatted column compartment and detection type 1024-element photodiode array with wavelength accuracy ± 1 nm. Mobile phase used was acetic acid (0.5 %) and methanol. Column used was Phenomenex C18, 5μ ODS (3), 100 Å, 250 X 4.6 mm (Torrance, California, United States) and the temperature of the column was 30 °C. The flow rate for SDG analysis was 0.6 mL/min. Injection volume used was 20 μl. UV detection at 280 nm was used for SDG analysis.

The flow rate of the solvent was maintained isocratic. Preparation of the sample was done by taking 2.5 g of extrudate in a volumetric flask (50 mL) followed by addition of 40 mL of mobile phase. Vortexing was done for 10 min. Volume was made with the same. Aliquots (10 mL) were taken. 1 M HCl (2 mL) was added. Then the flask containing the mixture was kept on boiling water bath (95 °C) for 1 h dilution was done up to the mark with the addition of mobile phase. Filtration of the solution was done with a syringe filter (0.45 μm). Injection of 20 μl of the filtered solution was done into HPLC system.

Preparation of the stock solution of SDG standard was done by taking pure (5 mg) SDG in volumetric flask (50 mL). Mobile phase (100 ppm stock) was added to make the final volume upto 50 mL. Preparation of the standard working solution was also done by taking 0.1, 1.0, 2.5, 5.0 and 10 mL from stock solution. Volume was made upto 10 mL with the addition of mobile phase to make up the final concentrations as 1, 10, 25, 50 and 100 ppm.

Calculation:

$$SDG(g/100g) = \frac{(Instrument\, sample\, concentration\, X\, Volume)}{Sample\, weight(g)\, X\, 10000}$$

Chromatographic analysis of gamma oryzanol in optimized sample

In the optimized sample with gamma oryzanol fortification at both levels, retention of oryzanol was analyzed by following the methodology of Azrina et al. (2008). Quantification of oryzanol content was performed using HPLC system (HPLC Agilent 1260 series, Santa Clara, California, United States) having G1311B/C quaternary pump, G1329B autosampler, G1316B thermostatted column compartment and detection type 1024-element photodiode array with wavelength accuracy ± 1 nm. Separation of oryzanol components was done on Phenomenex C18, 5μ ODS (3), 100 Å, 250 X 4.6 mm (Torrance, California, United States) and the mobile phase was methanol:acetonitrile:isopropanol (45:50:5).

Detection of oryzanol components was done at 330 nm. Flow rate for oryzanol analysis was 1.0 mL/min. The flow rate of the solvent was maintained isocratic. Sample (2.5 g) was taken in a volumetric flask (25 mL). Mobile phase (15 mL) (methanol: acetonitrile: isopropanol) was added to it followed by centrifugation of the mixture at 3100 × g for 5 min. Separation of the liquid was done which followed by dilution up to the mark with mobile phase. Filtration of the solution was done by using a syringe filter and injected to HPLC. Preparation of standard stock solution was done by taking oryzanol standard (10 mg) in a volumetric flask (10 mL). Mobile phase was added to make up the volume to give a stock solution of 1000 ppm. From this stock solution, standard working solutions were prepared at final concentrations of 50, 100, 500 and 1000 ppm by diluting with the mobile phase. With these four

Table 1

Effect of storage period on the retention of incorporated bioactive compounds in optimized fortified extrudates during storage.

Samples	Units	Relative humidity (%)	Retention						
			No. of Days						
			0	15	30	45	60	75	90
B_1	g/100 g	43.26	2.48 ± 0.03^a	2.47 ± 0.01^a	2.45 ± 0.02^b	2.45 ± 0.02^b	2.43 ± 0.01^c	2.42 ± 0.01^c	2.40 ± 0.01^d
B_2			5.45 ± 0.04^a	5.44 ± 0.02^{ab}	5.43 ± 0.04^{bc}	5.42 ± 0.03^c	5.40 ± 0.01^d	5.38 ± 0.02^e	5.37 ± 0.01^e
L_1	mg/kg		172.61 ± 3.12^a	172.54 ± 1.78^a	172.52 ± 0.07^a	172.24 ± 1.04^a	167.45 ± 1.64^b	165.43 ± 0.99^c	165.23 ± 0.58^c
L_2			233.30 ± 3.03^a	232.65 ± 2.01^a	232.55 ± 2.10^a	232.47 ± 1.49^a	230.43 ± 1.23^b	226.78 ± 1.55^c	226.34 ± 1.16^c
O_1	g/100 g		1.07 ± 0.02^a	1.07 ± 0.01^a	1.06 ± 0.01^a	1.06 ± 0.01^a	1.04 ± 0.01^b	1.02 ± 0.02^c	1.00 ± 0.01^d
O_2			1.55 ± 0.01^a	1.54 ± 0.02^a	1.54 ± 0.01^b	1.54 ± 0.01^b	1.50 ± 0.02^c	1.48 ± 0.02^d	1.46 ± 0.01^e
B_1	g/100 g	52.60	2.48 ± 0.03^a	2.47 ± 0.02^a	2.45 ± 0.02^b	2.43 ± 0.01^{cd}	2.42 ± 0.02^d	2.38 ± 0.03^{ef}	2.37 ± 0.01^f
B_2			5.45 ± 0.04^a	5.43 ± 0.02^b	5.42 ± 0.01^{bc}	5.40 ± 0.01^{cd}	5.39 ± 0.01^{de}	5.38 ± 0.03^e	5.36 ± 0.02^f
L_1	mg/kg		172.61 ± 3.12^a	172.52 ± 1.05^a	170.50 ± 1.48^b	170.23 ± 1.23^b	166.20 ± 1.06^c	164.10 ± 1.55^d	163.45 ± 0.92^d
L_2			233.30 ± 3.03^a	232.45 ± 1.13^a	230.78 ± 1.59^b	228.78 ± 1.76^c	226.67 ± 1.11^d	225.56 ± 1.69^d	225.12 ± 1.96^d
O_1	g/100 g		1.07 ± 0.02^a	1.07 ± 0.02^a	1.06 ± 0.02^a	1.06 ± 0.01^a	1.03 ± 1.01^b	1.01 ± 0.02^c	1.01 ± 0.01^c
O_2			1.55 ± 0.01^a	1.54 ± 0.01^a	1.54 ± 0.03^a	1.54 ± 0.02^a	1.49 ± 0.02^b	1.49 ± 0.02^b	1.47 ± 0.03^c
B_1	g/100 g	84.36	2.48 ± 0.03^a	2.46 ± 0.03^b	2.44 ± 0.02^c	2.43 ± 0.02^c	2.40 ± 0.01^d	2.41 ± 0.02^d	2.36 ± 0.01^e
B_2			5.45 ± 0.04^a	5.42 ± 0.03^{bc}	5.43 ± 0.02^b	5.41 ± 0.02^c	5.42 ± 0.03^b	5.36 ± 0.01^c	5.30 ± 0.02^d
L_1	mg/kg		172.61 ± 3.12^a	172.45 ± 2.42^a	169.89 ± 1.76^b	168.78 ± 0.90^b	165.43 ± 1.10^c	162.80 ± 1.03^d	160.68 ± 2.00^e
L_2			233.30 ± 3.03^a	230.67 ± 1.52^b	228.78 ± 0.70^c	227.67 ± 1.81^c	225.56 ± 2.07^d	223.67 ± 1.04^e	220.78 ± 1.05^f
O_1	g/100 g		1.07 ± 0.02^a	1.03 ± 0.01^b	1.01 ± 0.01^c	0.98 ± 0.01^d	0.85 ± 0.02^e	0.82 ± 0.02^f	0.80 ± 0.01^g
O_2			1.55 ± 0.01^a	1.52 ± 0.01^b	1.50 ± 0.02^c	1.46 ± 0.02^d	1.42 ± 0.02^d	1.40 ± 0.01^e	1.30 ± 0.01^f
B_1	g/100 g	93.58	2.48 ± 0.03^a	2.45 ± 0.03^b	2.42 ± 0.02^c	2.40 ± 0.02^d	2.34 ± 0.02^e	2.27 ± 0.02^f	2.24 ± 0.03^g
B_2			5.45 ± 0.04^a	5.40 ± 0.02^b	5.38 ± 0.03^c	5.36 ± 0.02^d	5.34 ± 0.02^e	5.32 ± 0.03^f	5.26 ± 0.02^g
L_1	mg/kg		172.61 ± 3.12^a	172.52 ± 0.49^a	164.56 ± 1.01^b	162.67 ± 0.70^c	160.78 ± 1.08^d	158.95 ± 1.70^e	155.56 ± 1.03^f
L_2			233.30 ± 3.03^a	225.52 ± 2.14^b	220.54 ± 1.56^c	219.67 ± 1.02^c	216.78 ± 1.15^d	215.45 ± 2.08^d	212.56 ± 2.12^e
O_1	g/100 g		1.07 ± 0.02^a	1.02 ± 0.01^b	1.01 ± 0.02^b	0.98 ± 0.01^c	0.83 ± 0.01^d	0.80 ± 0.02^e	0.78 ± 0.02^f
O_2			1.55 ± 0.01^a	1.51 ± 0.02^b	1.48 ± 0.02^c	1.43 ± 0.01^d	1.40 ± 0.02^e	1.39 ± 0.02^e	1.28 ± 0.03^f

Values are means ± standard deviations (n = 3).

Values for a particular row followed by different letters differ significantly (p < 0.05), B_1: extrudates fortified with beta-glucan concentrate at low level, B_2: extrudates fortified with beta-glucan concentrate at high level, L_1: extrudates fortified with flaxseed lignan concentrate at low level, L_2: extrudates fortified with flaxseed lignan concentrate at high level, O1: extrudates fortified with gamma oryzanol concentrate at low level, O2: extrudates fortified with gamma oryzanol concentrate at high level.

concentration levels, a calibration curve was plotted between concentration and peak area. Linearity was evaluated. For the reliability of results, determination of limit of detection (LOD) and limit of quantification (LOQ) for oryzanol was also done.

Storage studies of the developed extruded product

Storage study was conducted in order to evaluate various quality changes in corn-rice flour based extrudates fortified with the three bioactive concentrates at low and high levels upto a time period of 3 months and were analyzed for moisture content, textural hardness, colorimetric values (*L**, *a** and *b** values) and retention of bioactive compounds. Samples (10 g) were packed in laminated pouches (LP) of polyester-aluminum-polypropylene which were sealed air tightly by using polyethylene sealing machine and were placed in desiccators under four relative humidities using saturated salt solutions such as potassium carbonate (43.26 %), magnesium nitrate (52.60 %), potassium chloride (84.36 %) and potassium nitrate (93.58 %). The desiccators were placed in temperature-controlled cabinets maintained at 25 °C and the samples were allowed to equilibrate until there was no distinct weight change (±0.001 g).The total time for removal, weighing, and putting back the sample in the desiccators was about 30 s to minimize moisture absorption from atmosphere during weighing. One packet of each packaged material was taken out from the desiccator at an interval of 15 days up to three months and analyzed for selected parameters.

Statistical analyses

The data reported were average of triplicates. The results were expressed as means ± standard deviation. The data were analyzed by a one-way ANOVA at a confidence level of 95% (p ≤ 0.05) using Duncan multiple range test. The analysis was performed by using Statistica.v.12. (Stat Soft India Pvt. Ltd. New Delhi, India).

Results and discussions

Retention of bioactive compounds in the optimized samples after extrusion

Analysis of beta-glucan content in the optimized samples

Beta-glucan content of the optimized extruded samples fortified with beta-glucan concentrate at low level (3 g/100 g) and high level (6 g/100 g) was estimated as 2.48 g/100 g and 5.45 g/100 g, respectively (Table 1). Hence it was retained up to 82.67% and 90.83% at low and high level of substitution, respectively. The corresponding losses may be attributed to thermal and shear stress resulting in degradation of beta-glucan during the extrusion process. Yao et al. (2011) analyzed beta-glucan content in extruded breakfast cereal made from four oat lines namely N979, IA95, Jim and Paul having beta-glucan content as 8.6%, 7.6 %, 5.3% and 5.9%, respectively. The beta-glucan content reported in extrudates obtained from these four oat lines came out to be 4.7%, 4.5%, 3.2% and 3.5%, respectively. Thus it was concluded that the beta-glucan content estimated in extruded cereal was found approximately 60 percent of their corresponding oat lines which was attributed to thermal and shear stress responsible for degradation of beta-glucan in oat flour.
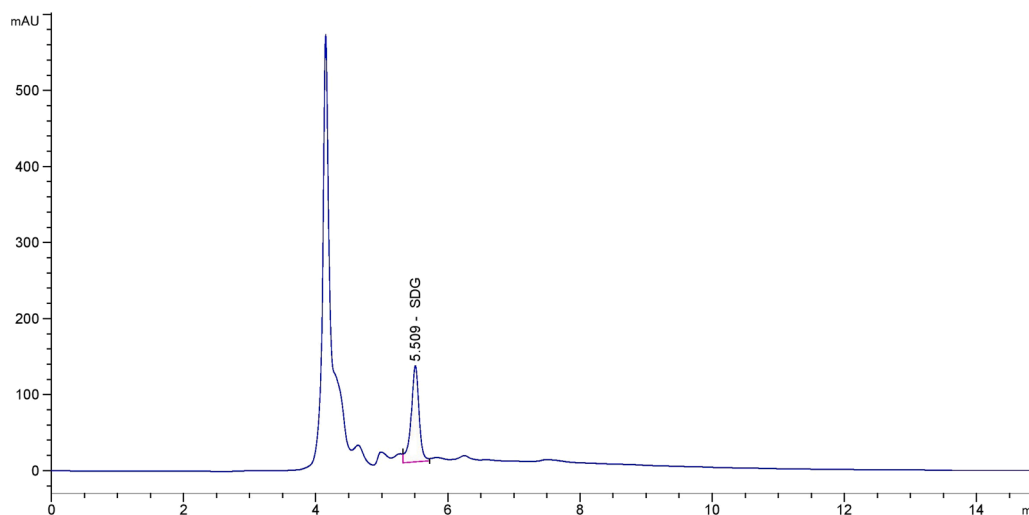
Fig. 1A. Chromatographic analysis of secoisolariciresinol diglucoside (SDG) in optimized sample with lignan fortification (at low level).

The current work exhibited much better results with respect to beta-glucan retention since extrudates were found to have 2.48% and 5.45% of beta-glucan content at substitution levels of 3% and 6%, respectively thereby having an estimated 82.67% and 90.83% of retention after extrusion processing.

There was a significant retention of beta-glucan content in extruded cereals made from four barley cultivars-Wanubet, Apollo, Bowman, Tupper and their blends with rice or wheat excepting the ones made from 100% Apollo and Wanubet-wheat blend (50:50) in which some loss in β-glucan content was observed as compared to their corresponding raw cereal mixes (Berglund et al., 1994). Alam (2012) also estimated beta-glucan content in extrudates made from defatted endosperm flour incorporated with the addition of defatted oat protein concentrate, defatted oat bran concentrate, ultra-fine oat bran concentrate and enzyme-hydrolyzed oat bran concentrate. Beta-glucan content of raw cereal mixes was found as 1.6%, 4.4%, 4.4% and 4.3% for whole endosperm flour, endosperm flour with the addition of 10 % oat bran concentrate, endosperm flour with the addition of 10% ultra fine bran concentrate and enzyme hydrolyzed oat bran concentrate, respectively. Beta-glucan content in extrudates came out to be 1.5%, 4.6%, 4.9% and 4.7%, respectively for their corresponding raw mixes. It was also reported that the highest level of beta-glucan content came out to be 7.1–7.4 % (d. b.) in extrudates with the addition of 20% oat bran

concentrate (30%, β-glucan content). Thus, there was a significant retention in beta-glucan content in extrudates made from endosperm flour with the incorporation of bran concentrates after extrusion process.

Delgado-Nieblas et al. (2019) reported that the extrusion process led to a decrease in beta-glucan content which was attributed to high temperature, pressure and shear during the production of breakfast cereals. An increase in soluble beta-glucan (SBG) was reported upon extrusion cooking which might be related with the shearing action of the extruder screw (Gaosong & Vasanthan, 2000) as well as temperature during extrusion (Brennan & Cleary, 2005). Repo-Carrasco-Valencia et al. (2009) also reported an increase in soluble fiber during extrusion and correlated it to high screw speed and high temperature leading to the production of smaller soluble molecules due to the cleavage of chemical bonds. On the other hand, Delgado-Nieblas et al. (2021) reported that the increase in soluble fiber was due to low moisture content, instead of high screw speed and barrel temperature during the extrusion process.

Chromatographic analysis of lignans (SDG) in the optimized samples

The optimized samples fortified with lignan concentrate at low (200 mg/kg) and high level (350 mg/kg) when subjected to HPLC analysis yielded SDG content quantified as 172.61 mg/kg (Fig. 1A) and 233.30 mg/kg (Fig. 1B), respectively. Hence the retention was 86.31% and
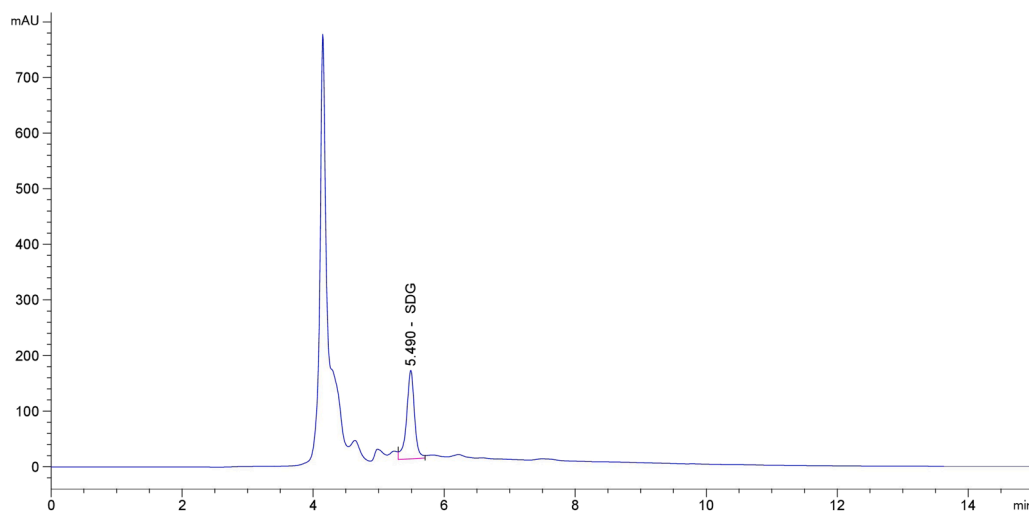

Fig. 1B. Chromatographic analysis of secoisolariciresinol diglucoside (SDG) in optimized sample with lignan fortification (at high level).
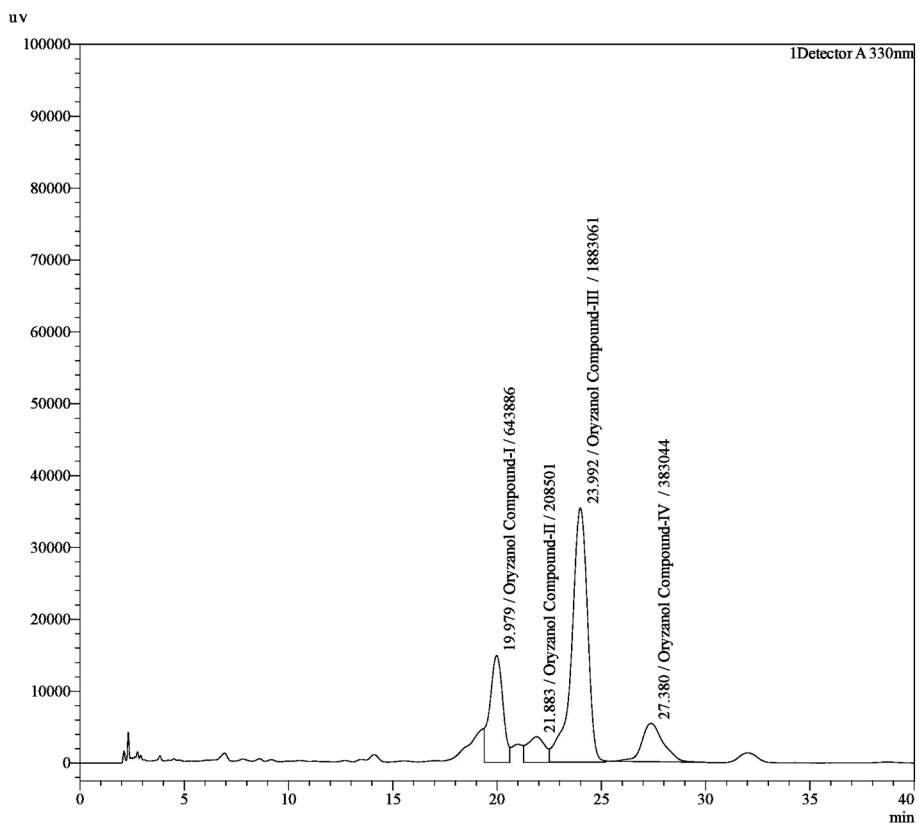
Fig. 2A. Chromatographic analysis of gamma oryzanol in optimized sample fortified with gamma oryzanol concentrate (at low level); Compound I: cycloartenyl ferulate; Compound II: 24-methylenecycloartanyl ferulate; Compound III: campesteryl ferulate; Compound IV: β-sitosteryl ferulate.
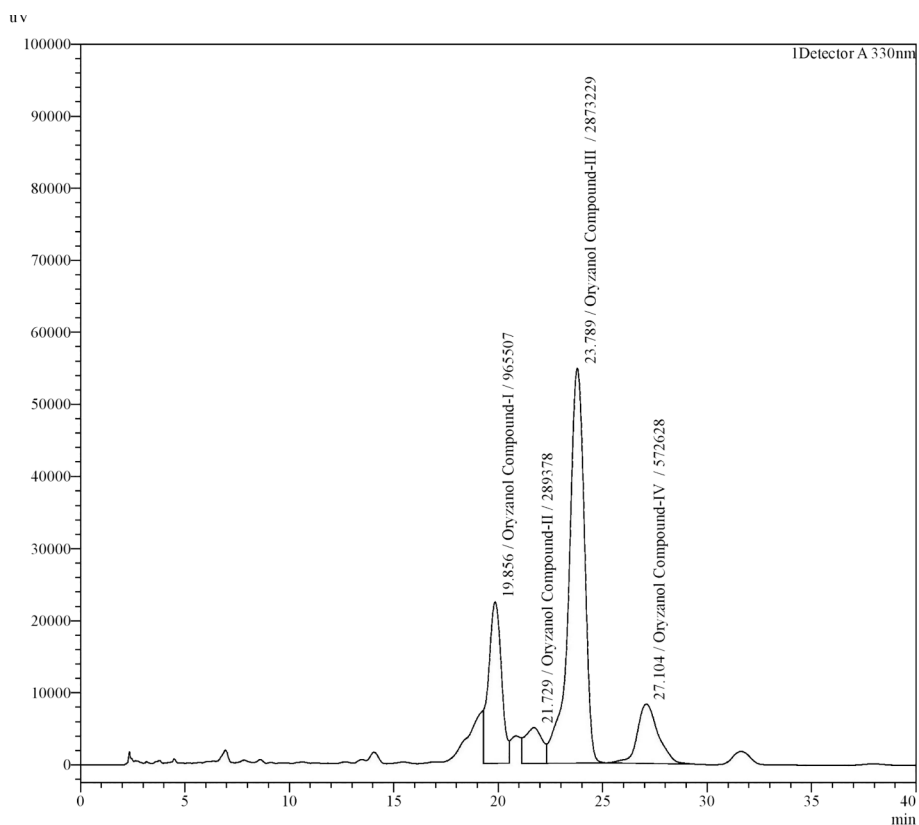

Fig. 2B. Chromatographic analysis of gamma oryzanol in optimized sample fortified with gamma oryzanol concentrate (at high level); Compound I: cyclo-artenylferulate; Compound II: 24-methylenecycloartanyl ferulate; Compound III: campesterylferulate; Compound IV: sitosteryl ferulate.

66.66% for low and high level, respectively. The loss of SDG after the extrusion process might be related to its entrapment in extruded cereal matrix leading to its inefficient extraction. Similar decomposition of SDG was reported in rye breads and muffin matrix which was related to the fact that SDG was bound to the bread matrix and muffin matrix as well which interfered with its extraction due to difficulties in analysis. Significant stability of SDG in all these baked stuffs was reported at baking temperature. Amount of SDG added to SDG buns was 300 mg and amount retained before proofing stage, after proofing and after baking was 278, 246 and 263 mg/kg (dw), respectively. SDG content in flax buns containing defatted flaxseed meal remained stable under storage in a freezer. In case of graham buns, SDG was found stable during proofing stage for 1.5 h. A relative stability of SDG was also reported during storage in muffins (Hyvärinen et al., 2006a). The findings of this work indicated that flaxseed derived lignans can be easily incorporated into bakery products which involves heating or baking with insignificant loss which is well in line with our work.

In another significant attempt to analyze the stability of incorporated lignans (SDG) in dairy food products such as heat treated milk, yoghurt mixture, edam cheese, whey drinks at a level of 200 mg, 100 mg, 1.0 g/ 10 L and 200 mg, respectively, it was reported that SDG content was not affected by heat processing. No degradation was observed due to fermentation caused by lactic acid bacteria and bifidobacteria in the yogurt starter signifying the stability of SDG towards hydrolysis by starter bacteria in comparison to soy isoflavones. Pressing and ripening did not affect SDG content in cheese. Apart from this, SDG was lost (25 %) during storage period of six months in whey drinks which was related to their low pH (3.0–4.1) (Hyvärinen et al., 2006b).

Muir and Westcott (2000) analyzed the stability of SDG in white, whole wheat, flax and multigrain breads with the inclusion of flax meal, alcoholic extracts of flaxseed and pure SDG into a white bread mix. With the addition of flax meal or alcoholic extracts, only 73–75% of the theoretical yield was obtained whereas 99.5% of SDG was recovered when pure SDG (82%) was incorporated to test loaves. Recovery of SDG was around 80–95% from macaroni fortified with flaxseed powder at levels that ranged between 10 and 20% indicating high stability of SDG during storage period of 32 weeks (Hall et al., 2005).

Chromatographic analysis of gamma oryzanol in the optimized samples

Gamma oryzanol content in the optimised fortified extrudates at low (1.5 g/100 g) and high (3.0 g/100 g) level was estimated as 1.07 g/100 g (Fig. 2A) and 1.55 g/100 g (Fig. 2B), respectively. Hence oryzanol was retained upto 71.33 % and 51.67 % for low and high level of fortification, respectively. Since gamma oryzanol concentrate isolated in the present study was a thick viscous mass which might not have blended and mixed well with the corn and rice flour blend leading to lower extractability. Another major reason behind less retention of gamma oryzanol might be due to the reason that during extrusion, gamma oryzanol concentrate being a thick viscous mass might has been bound to the extruded starch-protein matrix leading to its inefficient extractability during analysis. Gutierrez (2004) attempted on the incorporation of commercially available gamma oryzanol in cheddar cheese at a concentration of 100 mg /28 g of cheese and reported a decrease in gamma oryzanol during various stages of maturation which was related to improper mixing of gamma oryzanol which led to its reduced retention in cheese.

Retention of incorporated beta-glucans, lignans and gamma oryzanol during storage

The three bioactive concentrates incorporated were subjected to retention when kept under a storage period of three months at four different relative humidities. Each batch with low and high level of bioactive fortification was analyzed after a period of 15 days for three months. It was observed that under storage conditions of relative humidity (43.26%), beta-glucan content retained in optimized extrudates

fortified with beta-glucan content at both levels did not show any significant (p < 0.05) difference upto storage period of 15 days while as, a significant (p < 0.05) difference was seen after this storage period. Similar trend was observed under storage condition of relative humidity (52.60%). For storage conditions under higher relative humidities (84.36% and 93.58%), beta-glucan content retained in extrudates fortified with beta-glucan at both level of substitution revealed significant (p < 0.05) difference between 15 days throughout the storage time of three months (Table 1).

For extrudates fortified with lignans at both levels, it was seen there was no significant (p < 0.05) difference upto storage period of 45 days under relative humidity of 43.26%. Lignans retained in the fortified extrudates at both levels showed significant (p < 0.05) difference and comparatively lower retention after 45 days. Under relative humidity of 52.60%, lignans retained showed significant (p < 0.05) difference after a storage period of 15 days. Significant (p < 0.05) difference was observed in lignans retained throughout the storage period as lignan (SDG) content decreased when lignan fortified extrudates at both the levels were kept under storage condition of higher relative humidities (84.36% and 93.58%) (Table 1).

For gamma oryzanol fortified extrudates at both levels, oryzanol retention showed significant (p < 0.05) difference after storage period of 45 days with a decrease in oryzanol content when extrudates were kept under storage condition of lower relative humidities (43.26 % and 52.60 %) while as, significant (p < 0.05) differences were observed in oryzanol retention throughout the analysis period when a decrease in oryzanol content was found throughout the storage conditions of higher relative humidities (84.36% and 93.58%). Hence, it was seen that bioactive compounds retained showed significant (p < 0.05) differences when kept under storage condition of higher relative humidities (84.36% and 93.58%) as there was higher decrease in beta-glucan content, lignan (SDG) content and gamma oryzanol content as compared to the decrease observed at lower relative humidities (43.26% and 52.60%) (Table 1). This could be attributed to the ingression of high moisture under storage condition of higher relative humidities which could have led to the decomposition of beta-glucan, lignans (SDG) and gamma oryzanol in fortified extrudates at both levels as high moisture content in foods can be hazardous since it favours the growth of undesirable microorganisms.

Hyvärinen et al. (2006a) reported significant stability of SDG in bakery products such as graham buns and rye breads during storage period. An increase in SDG content was also observed during the storage period of two months at −25 °C. Retention and stability of SDG was also analyzed in various dairy products. In whey drinks, after a storage period of six months, certain loss in SDG was reported which was related to low pH of whey drinks. In yoghurt, SDG was found stable during storage period of 21 days at 4 °C (Hyvärinen et al., 2006b). There was also a significant difference in oryzanol content retained during storage period but with noticable retention (Gutierrez, 2004).

Storage studies of the optimized samples

Food products get exposed to various environmental factors such as temperature, humidity, etc. which can lead to several reactions resulting in degradation of food during the crucial stages of storage and distribution (Kumar et al., 2014). Factors affecting the keeping quality of several food products are a function of its environment surrounding it such as gaseous composition, relative humidity, mechanical stresses and temperature as well (Jan, Saxena, & Singh, 2017). Food products might be prone to damage to such an extent that they either face rejection by the consumer or they may lead to the production of harmful effects in them (Kumar et al., 2014).

Shelf life estimation has gained enormous attention due to consumer's reaction regarding the quality of the packaged products and it is the good storage stability of processed food which is a pivotal factor for enhancing utilization (Jan et al., 2017). Apart from extrinsic parameters

Table 2
Effect of storage on moisture content of extrudates fortified with bioactive compounds at two different levels.

Samples	Relative humidity (%)	No. of Days						
		0	15	30	45	60	75	90
B_1	43.26	6.51 ± 0.07[f]	6.52 ± 0.02[f]	6.54 ± 0.01[e]	6.56 ± 0.02[d]	6.61 ± 0.01[c]	6.63 ± 0.01[b]	6.70 ± 0.01[a]
B_2		6.65 ± 0.04[f]	6.67 ± 0.02[e]	6.69 ± 0.01[d]	6.72 ± 0.02[c]	6.75 ± 0.01[b]	6.76 ± 0.02[b]	6.78 ± 0.01[a]
L_1		6.60 ± 0.04[e]	6.63 ± 0.02[d]	6.68 ± 0.03[c]	6.71 ± 0.03[b]	6.72 ± 0.02[b]	6.73 ± 0.01[b]	6.75 ± 0.01[a]
L_2		7.36 ± 0.02[f]	7.38 ± 0.01[e]	7.42 ± 0.01[d]	7.44 ± 0.01[c]	7.45 ± 0.01[bc]	7.46 ± 0.01[b]	7.48 ± 0.02[a]
O_1		6.40 ± 0.03[g]	6.42 ± 0.01[f]	6.48 ± 0.01[e]	6.53 ± 0.01[d]	6.55 ± 0.04[c]	6.57 ± 0.02[b]	6.59 ± 0.01[a]
O_2		6.49 ± 0.01[f]	6.51 ± 0.02[e]	6.53 ± 0.02[d]	6.54 ± 0.02[d]	6.56 ± 0.01[c]	6.58 ± 0.01[b]	6.63 ± 0.01[a]
B_1	52.60	6.51 ± 0.07[g]	6.53 ± 0.02[f]	6.56 ± 0.02[e]	6.59 ± 0.02[d]	6.63 ± 0.01[c]	6.67 ± 0.03[b]	6.72 ± 0.02[a]
B_2		6.65 ± 0.04[g]	6.67 ± 0.03[f]	6.69 ± 0.01[e]	6.72 ± 0.02[d]	6.75 ± 0.02[c]	6.78 ± 0.02[b]	6.82 ± 0.01[a]
L_1		6.60 ± 0.04[g]	6.64 ± 0.017[f]	6.70 ± 0.02[e]	6.82 ± 0.03[d]	6.86 ± 0.01[c]	6.92 ± 0.03[b]	6.96 ± 0.01[a]
L_2		7.36 ± 0.02[g]	7.40 ± 0.02[f]	7.42 ± 0.02[e]	7.46 ± 0.01[d]	7.52 ± 0.01[c]	7.56 ± 0.02[b]	7.63 ± 0.01[a]
O_1		6.40 ± 0.03[f]	6.47 ± 0.01[e]	6.56 ± 0.02[d]	6.62 ± 0.01[c]	6.65 ± 0.02[b]	6.66 ± 0.02[b]	6.70 ± 0.17[a]
O_2		6.49 ± 0.01[g]	6.52 ± 0.02[f]	6.55 ± 0.02[e]	6.58 ± 0.01[d]	6.62 ± 0.02[c]	6.64 ± 0.01[b]	6.68 ± 0.02[a]
B_1	84.36	6.51 ± 0.07[g]	6.58 ± 0.02[f]	6.87 ± 0.02[e]	7.82 ± 0.02[d]	7.90 ± 0.02[c]	7.94 ± 0.01[b]	8.65 ± 0.02[a]
B_2		6.65 ± 0.04[g]	6.72 ± 0.01[f]	6.93 ± 0.02[e]	7.85 ± 0.02[d]	7.92 ± 0.01[c]	7.97 ± 0.01[b]	8.76 ± 0.02[a]
L_1		6.60 ± 0.04[g]	6.73 ± 0.01[f]	6.76 ± 0.01[e]	7.87 ± 0.01[d]	7.98 ± 0.02[c]	8.23 ± 0.02[b]	8.65 ± 0.02[a]
L_2		7.36 ± 0.02[g]	7.40 ± 0.03[f]	7.43 ± 0.01[e]	7.89 ± 0.02[d]	8.04 ± 0.02[c]	8.63 ± 0.02[b]	9.12 ± 0.02[a]
O_1		6.40 ± 0.03[g]	6.57 ± 0.02[f]	6.83 ± 0.02[e]	7.34 ± 0.01[d]	7.56 ± 0.02[c]	7.76 ± 0.01[b]	8.14 ± 0.01[a]
O_2		6.49 ± 0.01[g]	6.60 ± 0.1[f]	6.65 ± 0.01[e]	6.87 ± 0.01[d]	7.34 ± 0.01[c]	7.38 ± 0.02[b]	7.87 ± 0.01[a]
B_1	93.58	6.51 ± 0.07[g]	7.08 ± 0.02[f]	7.15 ± 0.02[e]	7.85 ± 0.02[d]	8.12 ± 0.02[c]	8.63 ± 0.01[b]	9.24 ± 0.02[a]
B_2		6.65 ± 0.04[g]	7.22 ± 0.01[f]	7.32 ± 0.01[e]	7.97 ± 0.01[d]	8.08 ± 0.02[c]	8.46 ± 0.02[b]	9.65 ± 0.01[a]
L_1		6.60 ± 0.04[g]	7.25 ± 0.02[f]	7.49 ± 0.02[e]	7.89 ± 0.02[d]	8.15 ± 0.01[c]	9.35 ± 0.01[b]	9.98 ± 0.01[a]
L_2		7.36 ± 0.02[g]	7.48 ± 0.02[f]	7.86 ± 0.01[e]	8.15 ± 0.01[d]	8.26 ± 0.02[c]	9.43 ± 0.01[b]	10.15 ± 0.0[a]
O_1		6.40 ± 0.03[g]	6.62 ± 0.02[f]	6.98 ± 0.02[e]	7.41 ± 0.01[d]	7.68 ± 0.02[c]	7.78 ± 0.01[b]	8.25 ± 0.02[a]
O_2		6.49 ± 0.01[g]	6.65 ± 0.01[f]	6.72 ± 0.02[e]	6.92 ± 0.01[d]	7.38 ± 0.01[c]	7.42 ± 0.01[b]	8.26 ± 0.01[a]

Values are means ± standard deviations (n = 3).

Values for a particular row followed by different letters differ significantly (p < 0.05), B_1: extrudates fortified with beta-glucan concentrate at low level, B_2: extrudates fortified with beta-glucan concentrate at high level, L_1: extrudates fortified with flaxseed lignan concentrate at low level, L_2: extrudates fortified with flaxseed lignan concentrate at high level, O1: extrudates fortified with gamma oryzanol concentrate at low level, O2: extrudates fortified with gamma oryzanol concentrate at high level.

such as temperature, packaging and relative humidity, the shelf life of the product is also determined by various intrinsic factors related to food such as oxygen availability, moisture content (water activity), pH value, additives and micro-organisms (Escobedo-Avellaneda, Velazquez, Torres, & Welti-Chanes, 2012). Longer shelf lives are reported for several products when storage studies were conducted at ambient conditions which make their experimental determination difficult (Muzaffar & Kumar, 2016). Therefore, storage study was conducted for the evaluation of quality changes and retention of bioactive compounds in corn-rice flour based fortified extrudates at both levels of substitution at 25 °C condition up to 3 months.

Moisture content

One of the crucial factors in the determination of shelf stability of

Table 3
Effect of storage on textural hardness of extrudates fortified with bioactive compounds at two different levels.

Samples	Relative humidity (%)	No. of Days						
		0	15	30	45	60	75	90
B_1	43.26	18.66 ± 0.05[a]	17.89 ± 0.01[b]	16.87 ± 0.01[c]	16.14 ± 0.02[d]	15.82 ± 0.02[e]	15.11 ± 0.01[f]	13.46 ± 0.04[g]
B_2		21.76 ± 0.01[a]	18.57 ± 0.02[b]	16.65 ± 0.02[c]	16.12 ± 0.02[d]	15.38 ± 0.03[e]	15.26 ± 0.02[f]	12.31 ± 0.01[g]
L_1		24.89 ± 0.02[a]	23.40 ± 0.03[b]	22.29 ± 0.03[c]	20.06 ± 0.03[d]	17.81 ± 0.03[e]	15.95 ± 0.02[f]	14.55 ± 0.03[g]
L_2		26.96 ± 0.01[a]	24.35 ± 0.02[b]	22.43 ± 0.01[c]	20.32 ± 0.03[d]	18.87 ± 0.03[e]	16.13 ± 0.02[f]	15.31 ± 0.03[g]
O_1		18.52 ± 0.03[a]	17.23 ± 0.02[b]	17.12 ± 0.02[c]	16.47 ± 0.01[d]	15.32 ± 0.02[e]	15.15 ± 0.01[f]	14.20 ± 0.02[g]
O_2		22.59 ± 0.02[a]	21.45 ± 0.03[ab]	20.16 ± 0.01[bc]	19.56 ± 0.01[cd]	18.79 ± 0.01[d]	18.21 ± 0.01[e]	17.15 ± 0.01[f]
B_1	52.60	18.66 ± 0.05[a]	16.49 ± 0.03[b]	15.56 ± 0.02[c]	14.78 ± 0.03[d]	14.30 ± 0.17[e]	13.89 ± 0.03[f]	12.45 ± 0.02[g]
B_2		21.76 ± 0.01[a]	17.56 ± 0.02[b]	16.45 ± 0.03[c]	15.15 ± 0.01[d]	14.10 ± 0.01[e]	13.47 ± 0.01[f]	12.59 ± 0.01[g]
L_1		24.89 ± 0.02[a]	22.35 ± 0.03[b]	21.70 ± 0.02[c]	19.99 ± 0.02[d]	17.71 ± 0.03[e]	16.18 ± 0.03[f]	14.41 ± 0.02[g]
L_2		26.96 ± 0.01[a]	22.27 ± 0.03[b]	21.70 ± 0.02[c]	20.81 ± 0.01[d]	16.67 ± 0.02[e]	14.22 ± 0.01[f]	12.72 ± 0.03[g]
O_1		18.52 ± 0.03[a]	16.71 ± 0.04[b]	15.10 ± 0.04[bc]	16.42 ± 0.02[b]	15.22 ± 0.05[bc]	14.47 ± 0.01[c]	12.38 ± 0.02[d]
O_2		22.59 ± 0.02[a]	20.48 ± 0.06[b]	19.28 ± 0.05[c]	19.17 ± 0.05[c]	17.35 ± 0.04[d]	16.67 ± 0.07[e]	15.29 ± 0.06[f]
B_1	84.36	18.66 ± 0.05[a]	16.67 ± 0.11[b]	14.71 ± 0.04[c]	13.24 ± 0.03[d]	11.72 ± 0.07[e]	9.63 ± 0.03[f]	7.24 ± 0.02[g]
B_2		21.76 ± 0.01[a]	19.23 ± 0.08[b]	16.24 ± 0.06[c]	14.73 ± 0.04[d]	12.21 ± 0.06[e]	10.65 ± 0.15[f]	8.87 ± 0.06[g]
L_1		24.89 ± 0.02[a]	21.56 ± 0.09[b]	18.26 ± 0.08[c]	16.44 ± 0.01[d]	14.24 ± 0.09[e]	12.35 ± 0.04[f]	7.54 ± 0.04[g]
L_2		26.96 ± 0.01[a]	23.24 ± 0.07[b]	20.14 ± 0.04[c]	17.58 ± 0.04[d]	14.81 ± 0.12[e]	11.27 ± 0.02[f]	9.52 ± 0.03[g]
O_1		18.52 ± 0.03[a]	16.63 ± 0.09[b]	14.18 ± 0.07[c]	12.29 ± 0.06[d]	10.35 ± 0.02[e]	9.29 ± 0.02[f]	7.52 ± 0.03[g]
O_2		22.59 ± 0.02[a]	19.86 ± 0.03[b]	18.37 ± 0.05[c]	16.79 ± 0.05[d]	14.41 ± 0.05[e]	12.25 ± 0.06[f]	10.73 ± 0.04[g]
B_1	93.58	18.66 ± 0.05[a]	15.44 ± 0.07[b]	13.50 ± 0.08[c]	11.69 ± 0.02[d]	9.85 ± 0.01[e]	7.81 ± 0.04[f]	5.84 ± 0.02[g]
B_2		21.76 ± 0.01[a]	16.20 ± 0.06[b]	13.30 ± 0.04[c]	11.56 ± 0.07[d]	9.12 ± 0.05[e]	8.60 ± 0.04[f]	6.65 ± 0.07[g]
L_1		24.89 ± 0.02[a]	17.16 ± 0.06[b]	14.36 ± 0.01[c]	10.41 ± 0.04[d]	8.16 ± 0.01[e]	6.81 ± 0.03[f]	5.29 ± 0.01[g]
L_2		26.96 ± 0.01[a]	18.10 ± 0.12[b]	14.87 ± 0.02[c]	11.24 ± 0.02[d]	8.24 ± 0.05[e]	6.21 ± 0.02[f]	4.94 ± 0.03[g]
O_1		18.52 ± 0.03[a]	15.24 ± 0.04[b]	12.74 ± 0.01[c]	11.27 ± 0.01[d]	9.65 ± 02[e]	8.95 ± 0.03[f]	6.21 ± 0.02[g]
O_2		22.59 ± 0.02[a]	16.85 ± 0.04[b]	14.31 ± 0.02[c]	11.85 ± 0.03[d]	10.23 ± 0.01[e]	8.98 ± 0.02[f]	6.82 ± 0.01[g]

Values are means ± standard deviations (n = 3).

Values for a particular row followed by different letters differ significantly (p < 0.05), B_1: extrudates fortified with beta-glucan concentrate at low level, B_2: extrudates fortified with beta-glucan concentrate at high level, L_1: extrudates fortified with flaxseed lignan concentrate at low level, L_2: extrudates fortified with flaxseed lignan concentrate at high level, O1: extrudates fortified with gamma oryzanol concentrate at low level, O2: extrudates fortified with gamma oryzanol concentrate at high level.

Table 4
Effect of storage period on color of extrudates fortified with beta-glucan concentrate at two different levels.

Sample	Relative humidity (%)	Colorimetric values	No. of Days						
			0	15	30	45	60	75	90
B_1	43.26	L*, a*, b*	82.97 ± 0.03[a]	82.45 ± 0.03[a]	81.47 ± 0.02[a]	80.23 ± 0.02[b]	79.29 ± 0.01[b]	77.39 ± 0.03[c]	77.35 ± 0.02[c]
			0.82 ± 0.01[f]	0.85 ± 0.02[e]	0.85 ± 0.01[e]	0.88 ± 0.01[d]	0.92 ± 0.01[c]	0.95 ± 0.01[b]	1.12 ± 0.02[a]
			22.57 ± 0.6[a]	22.54 ± 0.09[a]	21.67 ± 0.04[b]	20.55 ± 0.13[bc]	19.56 ± 0.06[c]	19.11 ± 0.03[c]	19.05 ± 0.04[c]
	52.60	L*, a*, b*	82.97 ± 0.05[a]	81.89 ± 0.02[ab]	80.78 ± 0.02[bc]	79.57 ± 0.01[cd]	78.97 ± 0.01[d]	76.89 ± 0.03[e]	75.45 ± 0.01[e]
			0.82 ± 0.01[f]	0.88 ± 0.01[e]	0.93 ± 0.02[d]	0.95 ± 0.01[c]	0.98 ± 0.01[c]	1.06 ± 0.02[b]	1.18 ± 0.01[a]
			22.57 ± 0.6[a]	21.69 ± 0.09[ab]	21.45 ± 0.04[ab]	20.18 ± 0.02[bc]	19.43 ± 0.02[cd]	18.57 ± 0.05[d]	18.32 ± 0.03[d]
	84.36	L*, a*, b*	82.97 ± 0.03[a]	80.67 ± 0.02[b]	78.72 ± 0.02[c]	78.54 ± 0.01[c]	75.46 ± 0.02[d]	73.65 ± 0.06[e]	71.87 ± 0.01[f]
			0.82 ± 0.01[e]	0.90 ± 0.01[d]	0.95 ± 0.01[c]	0.98 ± 0.01[c]	1.07 ± 0.02[b]	1.20 ± 0.02[a]	1.22 ± 0.02[a]
			22.57 ± 0.6[a]	21.97 ± 0.01[a]	19.76 ± 0.03[b]	18.53 ± 0.05[bc]	17.65 ± 0.04[cd]	16.87 ± 0.04[de]	15.23 ± 0.01[e]
	93.58	L*, a*, b*	82.97 ± 0.03[a]	81.12 ± 0.02[a]	76.47 ± 0.02[b]	75.56 ± 0.01[b]	73.49 ± 0.03[c]	70.32 ± 0.01[d]	65.12 ± 0.01[e]
			0.82 ± 0.01[f]	0.93 ± 0.01[e]	0.97 ± 0.02[d]	1.07 ± 0.03[c]	1.12 ± 0.02[b]	1.22 ± 0.03[a]	1.25 ± 0.03[a]
			22.57 ± 0.6[a]	20.48 ± 0.03[b]	19.87 ± 0.05[b]	17.43 ± 0.02[c]	16.64 ± 0.03[cd]	15.65 ± 0.03[de]	14.76 ± 0.02[e]
B_2	43.26	L*, a*, b*	80.48 ± 0.1[a]	79.89 ± 0.03[ab]	78.43 ± 0.06[b]	75.42 ± 0.1[c]	75.12 ± 0.04[c]	74.90 ± 0.04[c]	74.25 ± 0.03[c]
			0.85 ± 0.03[d]	0.86 ± 0.02[d]	0.90 ± 0.03[c]	0.95 ± 0.01[c]	1.06 ± 0.02[b]	1.12 ± 0.02[a]	1.11 ± 0.02[a]
			20.87 ± 0.03[a]	20.97 ± 0.04[a]	19.97 ± 0.04[ab]	18.56 ± 0.05[b]	18.45 ± 0.05[b]	18.11 ± 0.03[b]	18.05 ± 0.04[b]
	52.60	L*, a*, b*	80.48 ± 0.4[a]	78.85 ± 0.07[b]	78.23 ± 0.1[b]	76.87 ± 0.1[c]	75.96 ± 0.01[cd]	74.23 ± 0.08[de]	73.10 ± 0.01[e]
			0.85 ± 0.02[e]	0.89 ± 0.03[d]	0.92 ± 0.02[c]	0.91 ± 0.01[c]	1.10 ± 0.02[b]	1.20 ± 0.02[a]	1.21 ± 0.04[a]
			20.87 ± 0.01[a]	20.46 ± 0.04[a]	19.85 ± 0.03[ab]	18.23 ± 0.03[bc]	17.97 ± 0.03[c]	17.95 ± 0.04[c]	17.56 ± 0.03[c]
	84.36	L*, a*, b*	80.48 ± 0.4[a]	78.98 ± 0.07[b]	78.23 ± 0.1[b]	76.87 ± 0.03[c]	73.21 ± 0.03[d]	70.98 ± 0.02[e]	70.42 ± 0.03[e]
			0.85 ± 0.02[g]	0.91 ± 0.02[f]	0.98 ± 0.04[e]	1.02 ± 0.02[d]	1.20 ± 0.02[c]	1.25 ± 0.02[b]	1.31 ± 0.01[a]
			20.87 ± 0.03[a]	18.45 ± 0.02[b]	17.23 ± 0.05[b]	17.12 ± 0.03[b]	15.67 ± 0.06[c]	15.34 ± 0.07[c]	14.98 ± 0.03[c]
	93.58	L*, a*, b*	80.48 ± 0.4[a]	78.12 ± 0.1[b]	76.84 ± 0.07[c]	73.32 ± 0.05[d]	71.12 ± 0.07[e]	69.75 ± 0.05[f]	66.56 ± 0.09[g]
			0.85 ± 0.02[f]	0.92 ± 0.02[e]	0.95 ± 0.01[e]	1.06 ± 0.02[d]	1.21 ± 0.02[c]	1.32 ± 0.02[b]	1.45 ± 0.03[a]
			20.87 ± 0.03[a]	18.95 ± 0.04[b]	17.87 ± 0.02[bc]	17.65 ± 0.02[bc]	16.15 ± 0.02[cd]	15.98 ± 0.02[de]	14.45 ± 0.04[e]

Values are means ± standard deviations (n = 3).
Values for a particular row followed by different letters differ significantly (p < 0.05).
B_1: extrudates fortified with beta-glucan concentrate at low level, B_2: extrudates fortified with beta-glucan concentrate at high level.

food products is the moisture content. An overall increase in the moisture content of bioactive fortified extrudates at both levels was observed with respect to storage time with maximum increase observed under relative humidities-84.36% and 93.58%. Highest increase was seen in optimized extrudates fortified with lignan concentrate at high level as depicted in Table 2. The moisture content of gamma oryzanol fortified extrudates at relative humidity-93.58% ranged from 6.49 to 8.26% (w. b.) where for extrudates fortified with flaxseed lignans at high level, it ranged from 7.36% to 10.15% (w.b.). A similar increase in moisture content of extrudates and cookies made from *Chenopodium album* was observed during storage at ambient temperature for five months with more pronounced effects seen in LDPE bags as compared to laminated pouches (Jan et al., 2017). The increase in the moisture content was attributed to the hygroscopic nature of the product, packaging material and storage environment (Nagi et al., 2012). Similar trend was reported for breakfast cereals during a storage period of 6 months (Butt et al., 2004).

Textural hardness

Apart from moisture content, hardness is another factor which depicts the overall quality of the end product (Jan et al., 2017). A gradual decrease was observed in textural hardness of optimized fortified extrudates with more pronounced effect seen under relative humidity of

84.36% and 93.58%. The textural hardness of flaxseed lignan fortified extrudates at high level varied from 26.96 N to 9.52 N and 26.96 N to 4.94 N at relative humidity of 84.36% and 93.58%, respectively (Table 3). Hence a decrease in hardness was observed with increase in moisture content exhibiting an inverse correlation with higher ranges of moisture levels leading to cleavage of hydrophilic bonds resulting in loss of texture and unacceptable product. This is well in line with the findings of Wani and Kumar (2016).

Colorimetric values

Color is a major property which is perceived by the consumer (Kumar et al., 2014). There was an observable change in color in the extruded products with storage time (Wani & Kumar, 2016). A gradual decrease was observed in L* value of optimized fortified extrudates with much higher decrease observed when extrudates were kept under highest relative humidity (93.58%). This decrease in L* value indicated more darker extrudates with storage time which might be due to non-enzymatic browning which is similar to the findings of Wani and Kumar (2016) and Jan et al. (2017). An increase in a* value was observed in all optimized extrudates with higher increase at relative humidity (93.58%) whereas a decrease in b* value was observed in all the optimized samples with higher decrease seen at higher level of relative humidities (Table 4, Supplementary Tables 5 and 6). Increase in

a* value indicated more redness of the extrudates which was also correlated with the enzymatic browning of papaya cereal flakes and extrudates during storage studies (Sunita & Chauhan, 2008). Decrease in b* value was also well in line with the findings of Wani and Kumar (2016)and Kumar et al. (2014). Hence, during storage studies, it was concluded that with an increase in storage period and under higher relative humidities, much darker extrudates with an unacceptable color were observed.

Conclusions

Analysis of retention of bioactive compounds in the optimized samples after extrusion and during storage period has been discussed in this work. After extrusion, beta-glucan content of the optimized extrudates was retained up to 82.67% and 90.83%, lignan (SDG content) was retained up to 86.31% and 66.66% whereas the retention of gamma oryzanol was found as 71.33% and 51.67% at low and high level, respectively. The components of oryzanol detected in the sample were cycloartenyl feulate, 24-methylene cycloartanyl ferulate, campesteryl ferulate and β-sitosteryl ferulate.

Storage studies of the optimized samples was conducted for evaluation of moisture content, textural hardness, color and retention of beta-glucans, flaxseed lignans and gamma oryzanol at low and high levels upto a period of 3 months. It was observed that under storage conditions of lower relative humidities (43.26% and 52.60%), the bioactive compounds retention was higher during analysis period of 15 days whereas retention of bioactive compounds was lower when extrudates were kept under storage condition of higher relative humidities (84.36% and 93.58%). This could be related to the decomposition of beta-glucans, lignans (SDG) and gamma oryzanol in fortified extrudates at low and high levels due to the absorption of moisture leading to the degradation of bioactive compounds under higher relative humidity storage conditions.

A gradual decrease was observed in brightness (*L** value) of fortified extrudates with much higher decrease observed under highest relative humidity (93.58%). An increase in redness (*a** value) was observed in all optimized extrudates with higher increase at relative humidity (93.58%) whereas there was a decrease in *b** value in all the optimized samples with higher decrease seen at higher level of relative humidities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100191.

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