A peer-reviewed version of this preprint was published in PeerJ on 24 March 2020.

View the peer-reviewed version (peerj.com/articles/8788), which is the preferred citable publication unless you specifically need to cite this preprint.

Nisar N, Mustafa F, Tahir A, Qadri R, Yang Y, Khan MI, Wang F. 2020. Proximate composition, functional properties and quantitative analysis of benzoyl peroxide and benzoic acid in wheat flour samples: effect on wheat flour quality. PeerJ 8:e8788 https://doi.org/10.7717/peerj.8788

Proximate composition and quantitative analysis of benzoyl peroxide and benzoic acid in the wheat flour samples: wheat flour quality

Numrah Nisar[1]*, Faiza Mustafa[1], Arifa Tahira[1], Rashad Qadri[2]*, Yaodong Yang[3], Muhammad Imran Khan[4]

[1]Department of Environmental Sciences, Lahore College for Women University, Lahore, Punjab, Pakistan

[2]Institute of Horticultural Science, University of Agriculture, Faisalabad, Punjab, Pakistan

[3] Hainan Key Laboratory of Tropical Oil Crops Biology/Coconuts Research Institute, Chinese Academy of Tropical Agricultural Sciences, No.496Wenqing Av., Wenchang, Hainan 571339, People's Republic of China

[4]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan

Corresponding Author:

Numrah Nisar[1], Rashad Qadri[2]

[1]Department of Environmental Sciences, Lahore College for Women University, Lahore, Punjab, Pakistan

[2]Institute of Horticultural Science, University of Agriculture, Faisalabad, Punjab, Pakistan

Email address: numrah_nisar@hotmail.com; rashad.qadri@uaf.edu.pk

Abstract

Background.

Extensive milling processes have deprived wheat flour from essential nutrients. Objective of the current study was to assess the nutritive quality of commercial wheat flour (soft flour/SF) through analyses of proximate composition and functional properties as well as quantification of benzoyl peroxide (BP; added as bleaching agent in the SF).

Methods.

Test samples included commercial soft flour samples purchased from the local supplier from different flour mills (with additives) and a control sample without additives was prepared by grinding the seeds harvested from wheat crop grown in the experimental field of University of Agriculture, Faisalabad, under optimized field conditions without any fertilizer and insecticide. Benzoyl peroxide and Benzoic Acid quantification was performed through High Performance Liquid Chromatography

Results.

Results when compared with the whole wheat flour (WF; never received additives) indicated that SF had lesser fiber, protein and ash contents, whereas, higher damaged starch, fat, gluten and bulk density. A parallel experiment under selected conditions (temperature, time and solute concentration) showed dissociation of BP into BA soon after the exposure. Observed BA range (13.77 mg/g after 16hrs) in SF and exposure level assessment (44.3±1.36 mg/kg/BW) showed higher intake of BA on the consumption of SF. Results revealed superiority of WF over SF in nutritive qualities as well as free of toxicants such as BA.

KEYWORDS: *Benzoyl peroxide*; *Benzoic acid; Soft Flour*; *Whole Wheat Flour*; *High Performance Liquid Chromatography*

Introduction

Wheat is a principal cereal consumed world-wide in different forms, as a major food source. During milling process endosperm is milled (bran is separated) to obtain WF which is pale yellow tint that yields sticky dough. This dough normally does not bake well and if tried, bakery products it yields are less commercially acceptable. When the flour is stored, during natural maturation or aging, carotenoids undergo oxidation which reduces the colour and improves rheological properties of dough such as texture, loaf-volume as well as freshness retention (Liu et al. 2014). Lutein (a xanthophyll) is excessively found in wheat seeds (≈3mg/kg of dried wheat) and the yellow colour of the bread crumb is due to remains of lutein in wheat seeds (Mellado-Ortega & Hornero-Méndez 2017).

Wheat milling industries utilize oxidizing agents to accelerate the process of maturation and dough improvement. These agents oxidize sulf-hydryl groups in flour gluten protein that yields in increased stickiness of the wheat flour and making its appearance as SF (Onishi et al. 2004b). These oxidizing agents can be used for bleaching only, bleaching and dough improvement or dough improvement only. During flour bleaching conjugated double bond of carotenoids is disrupted to less conjugated colorless system, which gives flour a desirable texture for baking. One of the most commonly used oxidizing agent is BP which exhibits bleaching properties without influencing the baking or taste (Gaddipati et al. 1983). BP is a free radical initiator and it produces carotenoid oxidation like a typical free radical mechanism. No acceptable limits of BP have been specified in the regulations whereas it has been extensively utilized throughout the world as a bleaching agent without any recovery. In food processing BP is (> 92%) converted into BA. BA is mostly used as a preservative for soft drinks, fruits, juices and many other types of food. However, its higher concentration than allowable safety level (40 mg/kg) is harmful for humans. Its use as antimicrobial agent has long history for their use as antimicrobial agents. Acute toxicity is unknown, however, a sensitive person consuming BA more than 5 mg/kg of BW per day can confront non-immunological contact (pseudo-allergy) reactions, hyperpnoea, metabolic acidosis and convulsions (Wei et al. 2006) (Liu 2007).

Beside addition of BP, the SF is processed several times compared to the WF to achieve the fiber free fine particles for the improved texture of the bakery products. During this processing structure, sensory qualities, protein contents, fiber contents and nutritional components get severely affected. Antioxidants in WF, which are present in the germ and bran (Isabelle & Andre 2006), are mostly

removed in the refined flour. Therefore, the current study was designed to observe and compare the proximate composition and functional properties of SF and WF as well as BP and BA concentrations in them. Current study also followed the flour bleaching reaction through quantification of BP and BA at varied time intervals after exposing WF (without additives) to BP as a bleaching agent. The findings will also quantify the level of BA in SF which may get consumed and nutritional deprivation as a result of intensive grinding and sieving of wheat flour during milling.

Materials & Methods

Sample collection

Test samples included commercial soft flour (n=4, SF) samples purchased from the local supplier from different flour mills (with additives) and a control sample (n=1, WF) without additives was prepared by grinding the seeds harvested from wheat crop grown in the experimental field of University of Agriculture, Faisalabad, under optimized field conditions (16/8 D/N; 23±1°C; 14 Inch water) without any fertilizer and insecticide. All the flour samples were passed through sieve (75µm size) before packing them into air-tight plastic containers.

Determination of functional properties

Bulk density of flour was determined as described (Jehu-Appiah et al. 2011) by following ASTM D1895B prescribed procedure. The ground and sieved sample was allowed to flow freely in a circular container (0.615 L) with a suspended funnel of opening diameter (1.5 cm). The height of funnel was kept about 20 cm and the powder was stirred continuously to avoid clogging inside the opening. Container with the sample was dropped few times from the height of 150 mm to allow settling and release of air. Weight of the container with the sample was determined and weight/volume (loose bulk density) was obtained. Density was determined through the formula of d=m/v, where mass (g) is the weight of the sample and volume (ml) is the volume of the material. Water and oil absorption capacity (WAC and OAC) were determined through the method described (Beuchat 1977). For this, 1g of sample was allowed to mix with 10ml of distilled water for about 30 seconds. Sample was then allowed to stand at room temperature (25 ± 2 °C) for next 30 minutes and then centrifuged at 3000 rpm (30 min.). Volume of the supernatant was determined and WAC (mg/ml) was calculated by formula WAC=Vinitial-Vfinal, where V is the volume of

water (ml). Similar procedure was repeated for OAC determination using commercial cooking oil the absorbing agent. Emulsifying stability and activity (ES and EA) were determined by (Neto et al. 2001) described method with certain modifications. About 5 ml of flour dispersion (10 mg/ml of water) was homogenized with 5 ml of oil for 1 min. through vigorous shaking. The emulsion was then centrifuged (Sigma 2-6, Germany) at 1100 rpm for 10 min. Height (cm) of emulsified layer (ELH) was deducted from the total height of the tube contents (TC) to estimate the EA, EA=(ELH/TC)×100. ES was obtained by heating the emulsion at 80°C for 30 min. before centrifuging at 1300 rpm for 10 min. ES was then calculated by ES=(ELHA/TCA)×100, where ELHA is the height of emulsified layer after heating and TCA is total content of the tube before heating. The effect of concentration of EA and ES was determined by varying the capacity. The method established by (Coffmann & Garciaj 1977) for determination of foaming stability and capacity (FS and FC) of flours was employed in the current study. About 100 ml of distilled water was mixed with 10 g of flour. The suspension was mixed vigorously for 5 min. on magnetic stirrer (Irmco MSC Digital, Germany). The initial solution volume V1 and the final solution volume V2 were recorded (graduated cylinder). Foaming Capacity (FC) was also calculated from the formula FC = ((V2-V1)/V1) ×100. Foaming Stability (FS) was also determined by the foam volume that left after 8 hours and expressed as of initial foam volume (percentage). To determine the gelation properties (Least gelatinization concentration LGC and gelatinization temperature GnT), distilled water sample suspensions (2-10% w/v) were prepared. About 10 ml of these dispersions were transferred into test tubes. In boiling water bath these test tubes were heated for 1 hour then cooled for 2 hours at 4°C in a refrigerator. However, least gelation concentrations were taken when the samples did not fall from the inverted test tubes.

Proximate composition

Proximate composition was determined through FT-NIR spectroscopy (Burker-TENSOR 37 FTIR spectrometer, Germany) as well as conventional methods to deduce the comparison. These properties included moisture content (MC), ash contents (AC), crude protein (CP), gluten and starch contents. Other properties such as crude fiber and fat (CFF) were determined through solvent extractions.

Since the bulk density varies with MC, therefore, it was determined through ASABE standard S358.2 (Theerarattananoon et al. 2011). During this procedure, 100 g of sample was dried in a

forced air convection oven (IM-115, Germany) at 103 °C for 24 hours. The sample was then weighed on digital balance (0.01 g precision; TE-313S-DS, Germany) and MC was calculated through MC=Winitial - Wfinal. MC was also determined through FT-NIR spectroscopy. Flour samples of different moisture level were utilized to develop the model for calibration and multivariate analysis was conducted after gathering their spectra. Unknown sample was then analyzed against the calibration to give the moisture contents. Crude protein was determined through semi micro-Kjeldahl method (AACC adopted method 46-13; American association for clinical chemistry, 1995) and auto protein analyzer (Kjeltec 2400 auto-analyzer, Hillerod, Denmark). Here 1 g of flour sample was used along with keeping nitrogen to protein conversion factor of 5.7. AACC method 38-12 was utilized to determine gluten contents of the selected flour samples (25 g each).

Analytical method

NIR Omega G Analyser (Bruins Instruments, USA) was employed to analyze different parameters (protein, starch, fat, moisture, gluten) of the flour and grain samples. The spectral transmissions range was 700-1100 nm with 5 nm scan increment, measured at controlled room conditions of 24±1°C, RH 34±2%.

MIR spectroscopy

The FTIR transmission spectra were recorded at Burker-TENSOR 37 FTIR spectrometer with Michelson interferometer. Working range of the spectrometer was 4000-12000 cm-1 and spectra generated were interpreted on the basis overtones of different functional groups in the product. Resolution of spectrometer was 4 cm-1(max scan interval value was 2 cm-1) with maximum scan time kept at 5 seconds. MIR spectra were recorded at Opus 6.0 Burker Software using attenuated Total Reflectance (ATR) unit. The reference spectrum (empty sample bottle) was utilized as background measurement before loading in sample's spectra. About 8-10 g of sample in a sample bottle was utilized to generate the spectra in diffused reflectance mode. Three spectra per sample were recorded by rotating the sample bottle at 120°. The measurements were carried out under the controlled conditions of temperature 24±1°C, RH 34±2%. Each spectra were the average of 3 scans per object.

HPLC Determination

Sample preparation and bleaching reaction

For quantification of BPO and BA separate procedures were carried out. About 50g of flour was blended with 1.5mg of BPO (bleaching agent) to achieve a concentration of 30µg/g. The mixture was passed through polyester sieve (400 mesh/cm2) to attain homogeneous blend and held in dark. The bleaching reaction (performed at room temperature) was monitored after every hour. The product of reaction was extracted every hour for a period of 8 hrs, finally a sample at 12 hrs and 16 hrs. Then samples were analysed through HPLC and compared with the commercial standards and calibration graph for the quantification. Associated peaks (matching with the standard) were considered for generation of results.

Calibration Graph for BPO

Standard stock solution (SS) was generated by dissolving pure BP (60mg/L) in diethyl ether (100% purity) and working standards were obtained by diluting SS with appropriate volume of diethyl ether. For BA stock solution, pure compound (100mg) was dissolved in 100mL of methanol and working standards were prepared by diluting the stock solution. Calibration curve was then generated by plotting the absorption peaks against concentration.

Extraction Procedure for BPO and BA.

The standard procedure was carried out (at room temperature) in a flask with grinding stopper. About 100mL of diethyl ether was added to 50g of flour (already mixed and sifted with bleaching agent). This mixture was shaken vigorously on a magnetic stirrer for 10 minutes and left to settle for 15 minutes. Upper layer of this solution (containing the products of reaction) was withdrawn through the pipet and transferred into Falcon polypropylene tube (10mL) and held into ice until HPLC analysis.

HPLC method

The supernatant was analyzed by Waters 600 HPLC system at Inertsil ODS-80A column (5 µm, 4.6 x 250 mm; GL science Tokoyo, Japan) equipped Inertsil ODS-3 guard column (10mm×4mm i.d.) and Waters 2996 Photodiode array detector. The detection wavelength was kept at 235nm and column oven at 40°C. For isocratic separation the conditions were as follows: Water (Solvent A),

ACN (solvent B) and Benzoic acid (Solvent C); 55% B:45%A as mobile phase for 1mL/min. The gradient conditions for analysis were as follows: Water-glacial acetic acid (1000:1) (Solvent A), ACN-glacial acetic acid (1000:1) (solvent B); 18% B (10 min) was increased to 60% B (11-15 min hold) at flow rate 1.2 mL/min and column temperature 35°C.

Estimation of dietary intake

The mean dietary intake for both BP and BA was estimated to determine the exposure rate. For this purpose, 200 subjects (random sampling) were evaluated for their preferences of WF and SF as well as amount of daily intake through a questionnaire survey. The SF brands, which were reported to be consumed, were evaluated for the presence of BA. Further calculations were accomplished through the following equation:

$$Y=\sum(Xv\times Cv)/Bw$$

Where; Xv=average daily amount (kg) of wheat flour consumed by a subject

Cv = concentration of BA (mg/kg) as determined through HPLC in particular wheat flour sample

Bw = Average body wt. of the subject

Statistical analysis

All analyses were conducted in triplicate, therefore standard error of mean (SEM) was applied using the Statistixl 1.9 Add-in package within Excel. Two-way analysis of variance (ANOVA) was conducted on the data sets as obtained through four different samples and run simultaneously to develop a comparison. The aim was to give the significant difference in the data sets from different flour samples which was not achievable through univariate or one-way ANOVA.

Results

Both the commercially available soft flour (SF) and whole wheat flour (WF) were compared for their functional properties, emulsifying properties, foaming capacity, gelation capacity, proximate composition and benzoyl peroxide composition. Finally, the exposure of benzoyl peroxide was compared with the daily intake capacity to observe the exposure of consumers when they are consuming SF or WF. Detailed results are as under:

Functional Properties

The oil absorption capacity (OAC), as presented in the Table 1, showed that the SF sample has highest lipophilic tendency of 2.87 mL/g. Highest OAC (188% compared to 146% for WF) and WAC (408% compared to 140% for WF) was observed for SF4 and highest WAC was also observed for the same. Different flour mill samples had almost similar results for OAC.

Water Absorption Capacity (WAC) was also higher (140%) for WF compared to SF (<123%). Results revealed that emulsifying Activity (EA) was higher for WF (43.7%) whereas stability (ES) is higher for all SF samples (<42%). EA and ES of the WF and SF were observed to vary with the process of milling. The emulsifying properties vary inversely, therefore WF had highest EA and lowest ES. Foaming Capacity (FC) and Foaming stability (FS) collectively form the foaming properties of any flour. Both of the properties are directly proportional to one another, which were observed to be higher for WF (12.9% FC and 1.94% FS). FC and FS of WF is more (<12% and <1% respectively) compared to all SF samples (>9% and >1% respectively). A highly significant difference ($P < 0.001$) was observed when values were compared statistically with WF.

Gelation capacity

Gelation capacity (including gelatinization temperature GnT and least Gelatinization concentration LGC) is attributed and controlled by the balance between hydrophilic interactions and repulsive electrostatic interactions between the water/oil and proteins (Casanova et al. 2008). Results (Table 1) showed that WF has higher gelation capacity (GnT=59.21°C; LGC=8%) compared to all SF samples ($P<0.001$ when datasets were compared with the WF dataset). It can also be observed that both considered parameters for gelation capacity are directly related to each other such that increase in one also shows increase in the other.

Proximate composition

The proximate composition (moisture, crude fiber, fat, ash, starch, damaged starch) of all the flour samples is as summarized in the Table 2. The moisture content of SF is less (3.84-4.25%), protein contents of WF were higher (8.9% compared to 4.6% for SF) and total starch was also more for WF (76.92% compared to 50.21% for SF). The results indicated that milling process has detrimental effect on several properties of the WF. Most of the components such as crude protein,

gluten, damaged starch datasets showed highly significant difference (P<0.001) with the WF dataset. This shows that quality of SF was deteriorated while processing and refining.

Benzoyl peroxide concentration

BP and BA were determined simultaneously using gradient analysis (Table 3). The retention times were observed to be 17.5 min for BP and 7.8 min for BA (Fig. 1 & 2). The maximum absorption of BP was at 195 and 235 nm, however, a wavelength of 235 nm was used for measuring the compounds considering the possible interference from the food ingredients. The calibration curve had excellent linearity within the range of 0.05-16 µg/g for BP and 0.2-15 µg/g for BA. When run on isocratic gradient with 55% ACN, an excellent linearity was obtained for BP, however BA was not detected. Therefore, measurements at gradient conditions were further processed to generate the comparison.

BP is a free radical initiator and therefore, it causes the oxidation of carotenoids by free radical mechanism. The process (Fig. 3) leads to the formation of benzoic acid as a by-product (Saiz et al. 2001; Shan et al. 2007; Sumnu & Sahin 2008). No BA or BP were observed for the Whole Wheat Flour sample (WF) (Fig. 2). In the controlled samples, 30 µg/g of BP was added to the wheat flour samples and 99.5% recovery (29.5 µg/g) was observed soon after adding. After 3 hours of bleaching, the amount of BP reduced significantly to 4 µg/g (Fig. 4) that reached to zero after 8 hours. The contents of BA were observed to be 2.84 µg/g as recovered soon after the addition. This quantity increased to 8.9 µg/g after 3hr and increased further to 13.5 µg/g after 8hr. The contents of BA were determined again after 12hours though very small increase in quantity was observed (13.75 µg/g), which shows that the process of conversion got stabilized with slight variation. To confirm this, the floor was analyzed again after 16 hrs and quantity of BA didn't change much (13.77 µg/g). Local standards for maximum acceptable quantity have not yet been specified however, international standards (60 µg/g for BP; 0-5 mg/kg for BA as per body wt. by JECFA acceptable daily intakes) were considered in the current study. Analysis of WF and SF available commercially for BP and BA showed higher rates of BA in the SF samples (Table 4). This indicated higher amount of BA intake when SF based products are consumed.

Estimation of daily intake

Estimation of dietary exposure of BA through just wheat flour consumption was estimated (Table 5). Since flour is important component of people diet therefore it was deemed necessary to give the level of exposure through flour only. Rest of the food groups such as noodles and drinks, though unavoidable, also contain certain amount of BA but they were not considered in the current study. As evaluated through questionnaire study most of the considered subjects (72.5) were consuming SF as purchased from the flour mills.

HPLC was used for quantification of BP in both white and whole wheat flour samples. BA was also determined because BP was decomposed into benzoic acid within limited days (Fig. 4). A research on bleaching agents including BP and BA by HPLC during bleaching process of wheat flour. The retention time of BP was 17.5 min and that of BA was 7.6 min. After 30 hours of bleaching BP concentration was 11 ppm. After 3 months its concentration was reduced to 6 ppm. These results demonstrated that when benzoyl peroxide added to flour their greater amount was decomposed into benzoic acid within limited days of treatment. The analytical results of present study showed that that retention time of BPO was 17.5 minutes and BA was 7.5 minutes at 235nm. In WF samples no content of BP and BA were found. In white flour samples BP content ranges from 6.6-21 mg/kg and BA content ranges from 13-28 mg/kg.

Discussion:

Whole wheat flour (WF) and Soft Flour (SF) were compared for their properties such as functional properties, emulsifying properties, foaming capacity, gelation capacity, proximate composition and benzoyl peroxide composition. The comparison was made to aware and understand the choice of flour in daily meals and their potential associated risks.

The oil absorption capacity (OAC) of the flour varies with the intrinsic properties such as amino acid composition, protein conformation, hydrophilic-hydrophobic balance of amino acids, steric factors as well as lipid and carbohydrate composition of a flour sample (Mao & Hua 2012). Results showed that SF samples had more non-polar side chains compared to WF which enhanced the oil binding capacity of SF and reduced its water binding capacity. Higher OAC (188% compared to 146% for WF; Table 1) represents that the flour can retain flavor and could have optimum uses in different food products such as bakery items. More hydrophobic sites as in case of SF (OAC >175%), as represented through OAC value, are important for bakery items in which fat absorption

is desirable (Chandra et al. 2015; Chassagne-Berces et al. 2011). Water Absorption Capacity (WAC) is important since it gives the capacity of a flour to have higher hydration capacity, lower WAC (<123%) in case of SF and higher in case of WF (140%) means that excessive grinding in the flour mills and sieving has resulted in the modification of protein structure. Another factor of higher WAC could be the fibers retaining the water. Reduction of theses fibers in case of SF has reduced its capacity to absorb water (Onipe et al. 2017; Shewry 2009). WAC is an important component since it allows the food to have sufficient water retention and transfer of this water upon consumption. The interaction of protein with water is usually determined through water hydration, holding, water retention and water imbibing. WAC favors another phenomenon called Water Hydration Capacity (WHC). WHC is a physical feature and describes the ability of flour structure to prevent water from being released from a protein structure. During food processing the protein structures are deteriorated which may influence the WHC of wheat as in case of SF.

Swelling, another important functional property referred to as spontaneous uptake of water by protein matrix, is indirectly related to WHC. Since SF is lower in WHC therefore its swelling capacity is more (>18 compared to 17.8 in case of WF), which ensures longer life of bakery items. Water retention is very important for protein functionality which determines the cationic, non-ionic and anionic polar sites of the protein molecules on the flour surface (Zayas 1997). Lower WHC means that proteins have less water binding capacity or they are “salted out” that takes place when the proteins are precipitated out. (Ramaswamy et al. 2013) showed that the higher water retention is associated with linear form of arabinan compared to its branched form. The branched form of arabinan is unhealthy for consumption, therefore WF should be the choice for consumption instead of SF.

(Ali et al. 2014) reported that lesser water retention properties are directly related to the damaged starch contents of the wheat. During milling process, the flour obtained comes from the endosperm which is rich in starch contents. The physical process of grinding during which the cylinders move closer and closer causes the starch granules to rupture. This results in the damaged starch contents and also has detrimental effect on the protein composition. The higher water retention is may be due to the absorption of water in the starch granules which also limit its propagation. In case of WF, the flour is produced through grinding the whole seed (endosperm and periphery) which is rich in pentosan, starch, ash and protein in more or less original form.

342 The emulsifying properties vary inversely with the insoluble protein fractions and polysaccharides
343 (Chove et al. 2001; Haruna et al. 2011). The unfolding of proteins when at interface water/oil
344 determines the Emulsifying Stability (ES) and Emulsifying Activity (EA). ES of protein is related
345 to the ability to minimize the interfacial tension between oil and water when in emulsion. Surface
346 activity is determined through the ability of protein to migrate, deploy, absorb as well as rearrange
347 at interface. The region of the minimum solubility of proteins (isoelectric region) was the region
348 of least soluble and minimum emulsifying capacity. The emulsifying properties vary with the two
349 effects 1) absorption of the protein at oil or water interface results in a substantial decrease in
350 interfacial energy 2) structural, electrostatic and mechanical energy barriers are caused by the
351 interfacial layer that opposes destabilization (Chaparro Acuña et al. 2012; Kumar et al. 2011). This
352 property makes both EA and ES opposite to each other. Higher EA of WF (>40%) makes it a
353 preferable choice compared to SF.
354 (Akintayo et al. 1999) showed that Foaming Capacity (FC) is associated with the flexibility of
355 protein molecules which reduces the surface tension as well as the globular protein which can
356 hinder surface denaturation, therefore, leading to a low FC (<9% in case of SF). The foaming
357 capacity indicates that proteins have active sites on the flour. Soluble proteins reduce the surface
358 tension when at interface between the fluid surrounding the molecules and air bubbles, which
359 blocks the coalescence. Protein molecules can also be deployed, which interact with each other to
360 give multilayer or film protein which increases the flexibility of air-liquid interface. This results
361 in harder foam due to unbreakable bubbles (Adebowale & Lawal 2003). Higher FC and Foaming
362 stability (FS) of WF indicates that protein structures are not denatured yet and they still carry the
363 capacity; however, in case of SF they seem to have lost their arrangement due to which reduced
364 foaming properties has been observed.
365 Protein gelation is very important in several vegetables and other food items. The effective
366 overlapping of the functional groups between adjacent protein moieties is very important for the
367 gel network formation. Higher gelation capacity (Gelatinization temperature) for WF indicates that
368 this may not be a good choice in bakery items.
369 The flour at the beginning of the process comes from the endosperm, which is rich in starch and
370 as it reaches the end of grinding, flour comes from periphery which is rich in ash, pentosan and
371 protein. During the milling process, the seeds pass through heavy grinders to attain fine powdered
372 flour. This flour is further sieved and final product, obtained after series of sieving and treatment

with BP, is packed and sold as commercial white flour used for bakery items. Most of the components such as crude protein, gluten, damaged starch datasets showed highly significant difference (P<0.001) with the WF dataset. This shows that quality of SF was deteriorated while processing and refining.

Flour mills consume benzoyle peroxide (BP) to improve the appearance and white colour of flour. BP is a free radical initiator and therefore, it causes the oxidation of carotenoids by free radical mechanism. The process (Fig. 3) leads to the formation of benzoic acid (BA) as a by-product (Saiz et al. 2001; Shan et al. 2007; Sumnu & Sahin 2008). (Onishi et al. 2004a) reported that Chigasaki Health Centre in Kanagawa Prefecture in Japan observed about 60-100 µg/g of BA in December 1999, which was found to be due to BP introduced in the food items; therefore BP was being decomposed into BA. Current study suggested that not all BP was converted into BA, therefore traces are still left in the sample which was in compliance with the findings of the previous studies (Onishi et al. 2004a; Ponhong et al. 2015). Further analyses of SF indicated that all samples had traces of BP and excessive amount of BA in them. Study by (Ponhong et al. 2015) indicated that not all BP could get converted to BA and slight amount of BA is also introduced during the bleaching process that helps in the initiation of conversion process. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has approved the quantity of BP allowable to be about 0-40 mg/kg which has also been approved by (Organization 2001) considering the requirement of whitening of flour. However, increased temperature during baking also speeds up the processes of metabolism of BP to it's by products (and BA in particular). The maximum allowable level for BA is 150 mg/L according to European community food safety regulations (EC, 1995), which means daily intake of 55.8 mg of BA per person or 0.8 mg/kg body weight assuming 70 kg of weight is allowed (Solomon et al. 2005). According to JECFA acceptable daily intake was 0-5 mg/kg for BA and benzoate. Intake of benzoic acid in sensitive persons, lower than 5 mg/kg of body weight per day has been observed to cause non-immunological contact reaction. A few studies have reported strong allergic reactions such as urticarial, pruritus and rhinitis to benzoic acid and benzoate exposure. According to current study, Table 5, SF consumers gain 44.3 mg/kg/bw of BA per day which is above maximum allowable intake (5 mg/kg/bw) compared to those who consume WF.

Wheat flour is an unavoidable commodity. This shows that white SF as produced out of milling process are contributors of excessive amount of toxic benzoic acid (BA) in the consumers' body.

Results of current study indicated very high amount of BA entering in human body upon consumption of SF. In China standard limit of BP is not exceed 60 mg·Kg1; the maximum content of BP in wheat flour is 80 mg/Kg in the US, in Japan 300 mg/Kg, and also 50 mg/Kg in United Kingdom. In China, permissible amount of BPO are 0.045, 0.05 0.06 g/kg according to standards of food additives regulation (Wei et al. 2006). According to Japanese regulations allow the use of diluted BPO (19-22% w/w) in wheat flour which is lesser than 0.30 g/kg. In France, the use of BP is strictly banned. In UK and USA the permitted level BP are 0.05 g/kg, 0.045 g/kg respectively (Saiz et al. 2001). These standard limits demonstrated that the concentration of BP in SF was within permissible limit, however when this value was calculated on the basis of Avg. Daily Intake (ADI) and Estimated Daily Intake (EDI) (Table 5) it was observed that acute level of BA is getting influxed into the bodies if SF consumption is continued. Current study showed an EDI of 78.3 g/day if a person consumes SF in the diet which is very higher compared to the allowable daily intake (ADI=0-5 mg/kg/day). Results revealed that regular consumption of SF has profound effect on human health, therefore WF should be used instead.

Conclusions

Wheat flour is one of the most important ingredients of the food being consumed most frequently. To improve the baking quality, SF is often used instead of WF. Increased demands of fine texture and bleached colour has led to the enhanced concentration of BPO being added to the flour. Extensive milling and increased BPO reduced the nutritive value of SF and enriched it with the toxicant such as BA (as degradation product of BPO). Among various parameters of flour quality, protein and ash content in WF sample was more than SF samples. Therefore, there is need to improve wheat flour quality being sold in the market by limiting the rate of BPO added as bleaching agent. HPLC analyses effectively demonstrated the dissociation of BPO to BA, which means that BA in SF was due to BPO added as whitening agent.

References

Adebowale K, and Lawal O. 2003. Foaming, gelation and electrophoretic characteristics of mucuna bean (Mucuna pruriens) protein concentrates. *Food Chemistry* 83:237-246.

433 Akintayo E, Oshodi A, and Esuoso K. 1999. Effects of NaCl, ionic strength and pH on the
434 foaming and gelation of pigeon pea (Cajanus cajan) protein concentrates. *Food*
435 *Chemistry* 66:51-56.
436 Ali R, Khan MS, Sayeed SA, Ahmed R, Saeed SMG, and Mobin L. 2014. Relationship of
437 damaged starch with some physicochemical parameters in assessment of wheat
438 flour quality. *Pakistan Journal of Botany* 46:2217-2225.
439 Beuchat LR. 1977. Functional and electrophoretic characteristics of succinylated peanut
440 flour protein. *Journal of agricultural and food chemistry* 25:258-261.
441 Casanova F, Nakamura A, Masuda H, Lima L, and Fialho E. 2008. Functionality of
442 phosphorylated vicilin exposed to chemical and physical agents. *Food Chemistry*
443 107:1138-1143.
444 Chandra S, Singh S, and Kumari D. 2015. Evaluation of functional properties of
445 composite flours and sensorial attributes of composite flour biscuits. *Journal of*
446 *food science and technology* 52:3681-3688.
447 Chaparro Acuña SP, Gil González JH, and Aristizábal Torres ID. 2012. Physicochemical
448 characteristics and functional properties of vitabosa (mucuna deeringiana) and
449 soybean (glycine max). *Food Science and Technology* 32:98-105.
450 Chassagne-Berces S, Leitner M, Melado A, Barreiro P, Correa EC, Blank I, Gumy J-C, and
451 Chanvrier H. 2011. Effect of fibers and whole grain content on quality attributes of
452 extruded cereals. *Procedia Food Science* 1:17-23.
453 Chove BE, Grandison AS, and Lewis MJ. 2001. Emulsifying properties of soy protein
454 isolate fractions obtained by isoelectric precipitation. *Journal of the Science of*
455 *Food and Agriculture* 81:759-763.
456 Coffmann C, and Garciaj V. 1977. Functional properties and amino acid content of a
457 protein isolate from mung bean flour. *International Journal of Food Science &*
458 *Technology* 12:473-484.
459 Gaddipati N, Volpe F, and Anthony G. 1983. Quantitative determination of benzoyl
460 peroxide by high-performance liquid chromatography and comparison to the
461 iodometric method. *Journal of pharmaceutical sciences* 72:1398-1400.
462 Haruna M, Udobi C, and Ndife J. 2011. Effect of added brewers dry grain on the physico-
463 chemical, microbial and sensory quality of wheat bread. *American Journal of Food*
464 *and Nutrition* 1:39-43.
465 Isabelle L, and Andre L. 2006. Quantitative prediction of microbial behaviour during food
466 processing using an integrated modelling approach: a review. *International*
467 *Journal of Refrigeration* 29:968-984.
468 Jehu-Appiah C, Aryeetey G, Agyepong I, Spaan E, and Baltussen R. 2011. Household
469 perceptions and their implications for enrolment in the National Health Insurance
470 Scheme in Ghana. *Health Policy and Planning* 27:222-233.
471 Kumar P, Yadava R, Gollen B, Kumar S, Verma RK, and Yadav S. 2011. Nutritional
472 contents and medicinal properties of wheat: a review. *Life Sci Med Res* 22:1-10.
473 Liu RH. 2007. Whole grain phytochemicals and health. *Journal of Cereal Science* 46:207-
474 219.
475 Liu Y, Zhao J, Li Z, Mu C, Ma W, Hu H, Jiang K, Lin H, Ade H, and Yan H. 2014.
476 Aggregation and morphology control enables multiple cases of high-efficiency
477 polymer solar cells. *Nature communications* 5:5293.
478 Mao X, and Hua Y. 2012. Composition, structure and functional properties of protein
479 concentrates and isolates produced from walnut (Juglans regia L.). *International*
480 *journal of molecular sciences* 13:1561-1581.
481 Mellado-Ortega E, and Hornero-Méndez D. 2017. Effect of long-term storage on the free
482 and esterified carotenoids in durum wheat (Triticum turgidum conv. durum) and

tritordeum (× Tritordeum Ascherson et Graebner) grains. *Food Research International* 99:877-890.

Neto VQ, Narain N, Silva J, and Bora P. 2001. Functional properties of raw and heat processed cashew nut (Anacardium occidentale, L.) kernel protein isolates. *Food/Nahrung* 45:258-262.

Onipe OO, Beswa D, and Jideani AIO. 2017. Effect of size reduction on colour, hydration and rheological properties of wheat bran. *Food Science and Technology (Campinas)*:0-0.

Onishi H, Araki T, Shirato H, Nagata Y, Hiraoka M, Gomi K, Yamashita T, Niibe Y, Karasawa K, and Hayakawa K. 2004a. Stereotactic hypofractionated high-dose irradiation for stage I nonsmall cell lung carcinoma. *Cancer* 101:1623-1631.

Onishi H, Araki T, Shirato H, Nagata Y, Hiraoka M, Gomi K, Yamashita T, Niibe Y, Karasawa K, and Hayakawa K. 2004b. Stereotactic hypofractionated high-dose irradiation for stage I nonsmall cell lung carcinoma: clinical outcomes in 245 subjects in a Japanese multiinstitutional study. *Cancer* 101:1623-1631.

Organization WH. 2001. *The World Health Report 2001: Mental health: new understanding, new hope*: World Health Organization.

Ponhong K, Teshima N, Grudpan K, Vichapong J, Motomizu S, and Sakai T. 2015. Successive determination of urinary bilirubin and creatinine employing simultaneous injection effective mixing flow analysis. *Talanta* 133:71-76.

Ramaswamy UR, Kabel MA, Schols HA, and Gruppen H. 2013. Structural features and water holding capacities of pressed potato fibre polysaccharides. *Carbohydrate polymers* 93:589-596.

Saiz A, Manrique G, and Fritz R. 2001. Determination of benzoyl peroxide and benzoic acid levels by HPLC during wheat flour bleaching process. *Journal of agricultural and food chemistry* 49:98-102.

Shan D, Shi Q, Zhu D, and Xue H. 2007. Inhibitive detection of benzoic acid using a novel phenols biosensor based on polyaniline–polyacrylonitrile composite matrix. *Talanta* 72:1767-1772.

Shewry P. 2009. The HEALTHGRAIN programme opens new opportunities for improving wheat for nutrition and health. *Nutrition Bulletin* 34:225-231.

Solomon K, Anadon A, Cerdeira A, Marshall J, and Sanin L. 2005. Informe preparado para la Comisión Interamericana para el control y el abuso de drogas (CICAD).

Sumnu SG, and Sahin S. 2008. *Food engineering aspects of baking sweet goods*: CRC Press.

Theerarattananoon K, Xu F, Wilson J, Ballard R, Mckinney L, Staggenborg S, Vadlani P, Pei Z, and Wang D. 2011. Physical properties of pellets made from sorghum stalk, corn stover, wheat straw, and big bluestem. *Industrial Crops and Products* 33:325-332.

Wei L, Zhujun Z, and Liu Y. 2006. Chemiluminescence microfluidic chip fabricated in PMMA for determination of benzoyl peroxide in flour. *Food Chemistry* 95:693-698.

Zayas JF. 1997. Solubility of proteins. *Functionality of proteins in food*: Springer, 6-75.

Figures

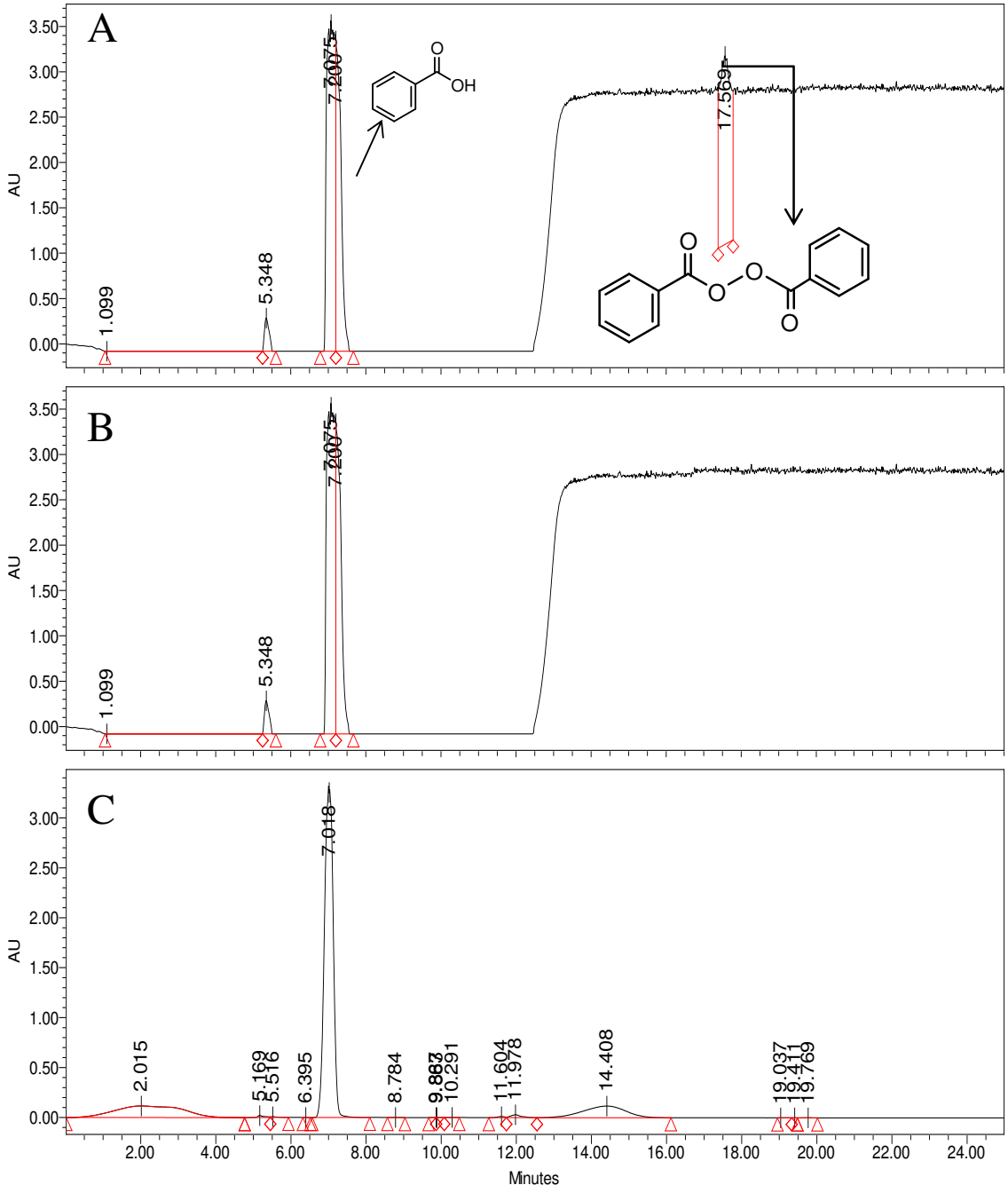

Figure 1: HPLC chromatogram of BP (17.56 min) and BA (7.018 min) in WF when induced with 30µg/g of BP. (A) shows the chromatogram of flour after 8hrs, (B) after 12hrs and (C) BA (7.018) in WF exposed to 30µg/g of BP after 12hrs through isocratic gradient elution.

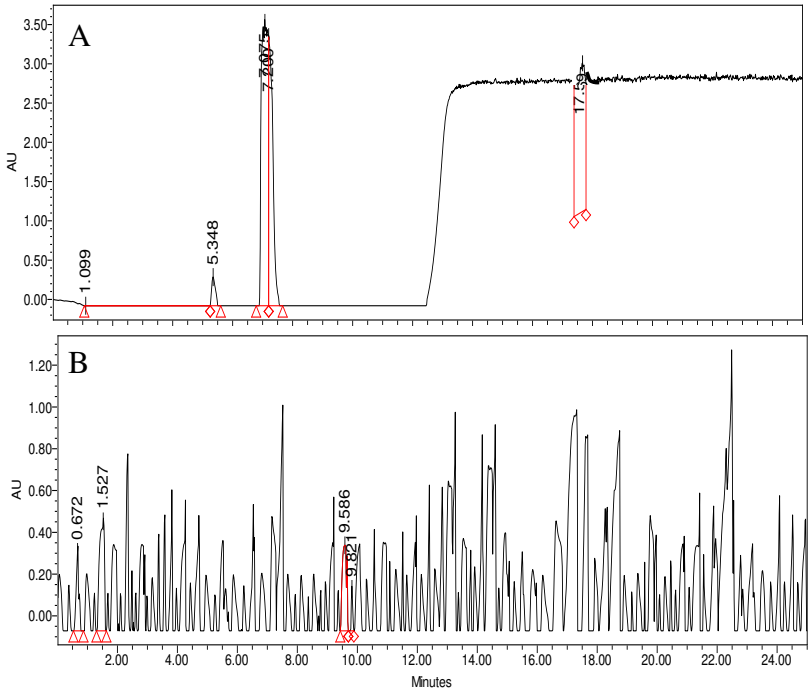

Figure 2: HPLC chromatograms for BPO and BA in A) commercial soft flour sample (SF1) B) whole flour (WF) without any addition of preservatives.

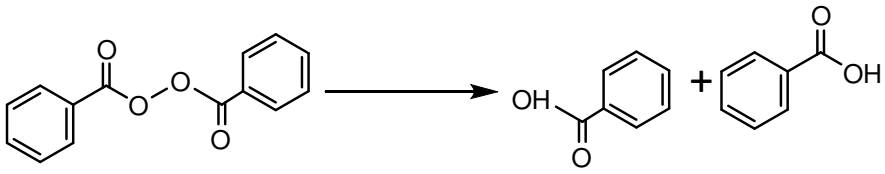

Figure 3: Possible pathway for the degradation of benzoyl peroxide (BP) to benzoic acid (BA) as observed through current research.

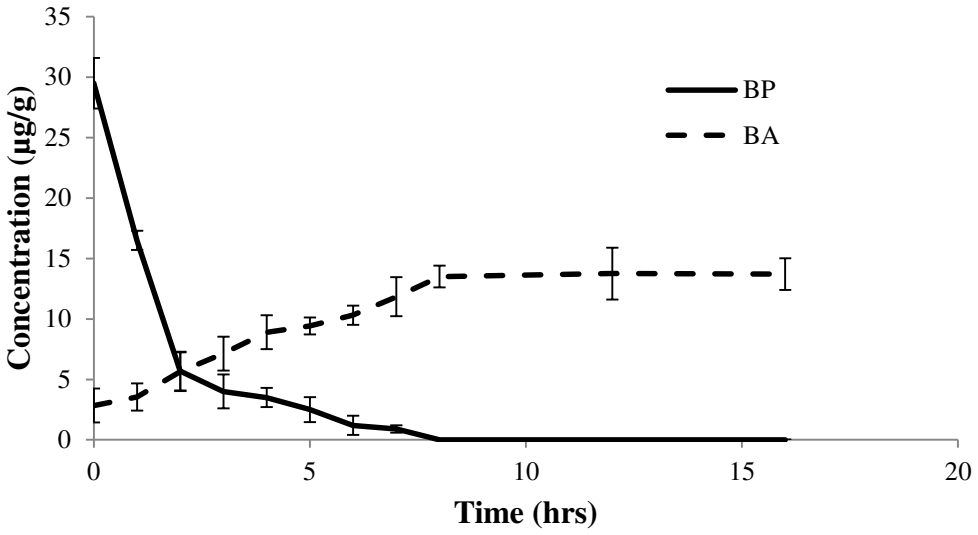

Figure 4: Rate of conversion of benzoyl peroxide (BP) to benzoic acid (BA) in flour at varied time intervals. About 50g of flour was amended with 30µg/g of BPO as bleaching agent. Rate of dissociation was observed over a period of 16 hrs.

TABLES

Table 1: Functional Properties of the whole wheat flour (WF) and soft flour (SF) samples (*P<0.05; **P<0.01; significant difference as calculated through ANOVA).

Samples	SC at 95°C	WAC (%)	OAC (%)	EA (%)	ES (%)	FC (%)	FS (%)	GnT (°C)	LGC (%)	BD (g/cc)
WF	17.8	140	146	43.7	38.4	12.9	1.94	59.21	8	0.76
SF1	19.4	121	181	37.84	44.6	8.97**	0.77	41.21*	6*	0.23
SF2	18.8	123	175	36.44	45.4	8.66**	0.84	38.7*	4**	0.24
SF3	19.6	120	184	38.45	43.5	8.71**	0.76	37.74*	4**	0.21
SF4	19.7	118	188	36.7	42.9	8.34**	0.74	38.45*	4**	0.26

Notes:

*SC=swelling capacity; WAC=water absorption capacity; OAC=oil absorption capacity; EA=emulsifying activity; ES=emulsifying stability; FC=foaming capacity; FS=foaming stability; GnT=gelatinization temp.; LGC=least gelatinization concentration; BD=bulk density

Table 2: Proximate composition of whole wheat flour (WF) and soft flour (SF) samples (*P<0.05; **P<0.01; significant difference as calculated through ANOVA).

Samples	Moisture (%)	Ash (%)	Crude fibre (%)	Fat (%)	Crude Protein (%)	Gluten (%)	Starch (%)	Sugar (%)	Damaged starch (%)	Pentosan (%)
WF	8.64	1.6	1.44	2.29	8.9	14.4	76.92	16.92	45.84	1.6
SF1	4.25*	0.54	0.34*	1.54	4.65**	7**	50.26**	23.41	88.36**	0.25
SF2	4.22*	0.55	0.34*	1.32	4.32**	7.5**	50.4**	23.15	87.22**	0.23
SF3	3.84**	0.45	0.32*	1.12	4.15**	6**	50.21**	23.4	82.3**	0.24
SF4	3.9**	0.34	0.33*	1.12	4.12**	6.5**	50.21**	23.25	85.62**	0.26

Table 3: Recoveries of Benzoyl peroxide (BP) and Benzoic acid (BA) from the flour.

Components	Added amount	Recovery (%)	
		Gradient	Isocratic
Benzoyl Peroxide	7	98.1±0.55	99.2±0.41
	30	99.5±0.61	99.3±0.55
	60	96±0.25	99.3±0.21
Benzoic acid	5	91.3±0.44	-
	10	91.3±0.31	-

Table 4: Concentrations of BP and BA in different flour samples

Samples	Contents (μg/g)	
	BP	**BA**
WW	0	0
SF1	2.45±1.1	68.11±14.1
SF2	3.41±1.02	71.4±12.31
SF3	2.54±0.94	71.51±15.84
SF4	5.77±0.33	72.55±15.33

Table 5: Estimated intake of BA on the basis of intake of flour by consumers.

Food group	Percentage Consumer (%)	Avg. Daily Intake (ADI) of flour (g/day)	Estimated Daily intake (EDI) of BA (mg/kg/bw)
SF	72.5	78.3±2.3	44.3±1.36
WF	10.5	72.1±3.3	1.34±1.45
Alternate	17	68.3±1.23	-

ADI for BA=0-5 mg/kg/bw