JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

CHANGES IN THERMAL PROPERTIES AND COLOUR ATTRIBUTES OF POTATO (CHANDRAMUKHI VARIETY) DURING FOAM MAT AND THIN LAYER DRYING

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doi: 10.15414/jmbfs.2017.6.5.1121-1126

ARTICLE INFO

Received 4. 11. 2014
Revised 26. 10. 2016
Accepted 16. 1. 2017
Published 1. 4. 2017

Regular article

OPEN ACCESS

ABSTRACT

Thermal properties of potato of Chandramukhi variety, including density, specific heat, thermal conductivity and thermal diffusivity during foam mat and thin layer drying were determined. The thin layer drying was conducted at three different temperatures i.e. 50°C, 55°C, 60°C. The foam mat drying was also conducted at three different temperatures (50, 55, 60°C) with three different concentrations of foaming agent (1%, 2%, and 3%). Glycerol monostearate (GMS) was used as a foaming agent. The specific heat, thermal conductivity and thermal diffusivity decreases as temperature increases from 50°C to 60°C in both of the drying. The foaming agent has a significant effect on drying rate and thermo-physical properties at p<0.05 level. Temperature and foaming both have a significant effect on color of dried powder at p<0.05 level. These thermal characteristics can be practically applied in modeling thermal behavior of potato mash during thermal processing operations.

Keywords: Chandramukhi variety potato, specific heat, thermal conductivity, thermal diffusivity, Hunter Lab colorimeter analysis

INTRODUCTION

Potato, a starchy, tuberous crop from the perennial Solanumtuberosum of the Nightshade family, is a major food crop in the world. It is a rich source of carbohydrate and its protein has a higher biological value than cereals and considered to be better than milk. Global potato production rate is about 324 million metric tons per year out of which India contributes to about 36.5 million metric tons per year as per a study undertaken in 2010. Due to inadequate storage facilities and processing units about 17-20% is wasted and due to its perishability more than 50% of surplus is wasted during transportation. Only about 5% of the world's potato crop is traded internationally (**Food Processing Industries Survey, West Bengal**). The export potential of potato can be increased by employing an ideal preservation technique that will increase the shelf life, diversify its usability, help in development of some innovative food products, as well as stabilize its international market value.

Thin layer drying is one of the most common methods used for preserving potatoes and extending their shelf lives by reducing the moisture content to a low level. Another comparatively new drying technique foam- mat drying, originally developed by Morgan et al. in 1959 at the Western Regional Research Laboratory of the U.S. Department of Agriculture (**Morgan *et al*, 1961**,), is a promising new development in the field of drying of high moisture foods. Drying of food material depends upon the heat and mass transfer characteristics of the product being dried. The thermal properties are unique and influence the efficiency of the process as well as the process itself involving changes in temperature of the process and composition of the food material (**Heldman*et al*., 2002**).

Thermal properties aid in selection and calibration of the drying equipments and understanding the transformations occurring inside the food matrix, e.g., the evolution of Biot number, the change in heating kinetics inside the particle as the drying progresses. Density, thermal conductivity, thermal diffusivity and specific heat capacity are four important thermal engineering properties of a material related to heat transfer characteristics. These parameters are essential in studying thermal processes of potato. Specific heat in thermal processes has a key role in computation of energy cost and also required dimensions for machinery and equipment design. Moreover, this parameter changes in food products along with their physical and chemical properties (**Mioe and Grodek, 2007**). Thermal conductivity of food materials is very important not only for process design, but also to predict and control of different changes occurring in food during thermal processing. Generally, this character depends on moisture content, temperature, porosity, nutritional content and component phase's distribution. Also, thermal diffusivity is a necessary transport attribute that is needed in modelling and computations of transient heat transfer in basic food processing operations, including drying, cooling/freezing and thermal processing (**Kocabiyik*et al*. 2009**).

Research on thermal properties has been reported for agricultural crops, especially different types of seeds, such as borage seed (Boragoofficinalis) (**Yang *et al*., 2002**), cumin seed (CuminumcyminumLinn.) (**Singh and Goswami, 2000**), guna seed (Citrulluscolocynthis) (**Aviara*et al*., 2008**), black seed (Nigella oxypetalaBoiss.) (**Gharibzahedi*et al*.**, 2012), roselle seed (Hibiscus sabdariffaL.) (**Bamgboye and Adejumo, 2010**) and Loco (Concholepasconcholepas) (**Reyes *et al*., 2011**).

This investigation has been carried out with the specific objective of determining the thermal properties of potato of Chandramukhi variety. Moreover incorporation of foam changes the structure of the food matrix which directly effects the transport processes during foam mat drying. Hence the effect of different degrees of foaming and moisture reduction under elevated levels of temperature on these properties was also studied. Colour as a physical property was determined as a measurement of the overall effect of the processing conditions on the quality and acceptability of the final dried sample.

MATERIALS & METHODS

Collection and Preparation of Raw Material

Potatoes used in this study were of Chandramukhi variety freshly collected from local market of South Kolkata (cultivated in Tarakeswar, Hoogly district, West Bengal, India). The potato samples were washed with running tap water and distilled water respectively to make it free from dirt and soil and blotted with a tissue paper for removal of excess surface water. The potato samples were then peeled and cut into slices of equal thickness of 10±0.3 mm each. The potatoes had initial moisture content of 82.34gm of moisture/100gm wet weight. The sliced potato samples were blanched in hot water (Temperature 90±20°C) containing 2 gm NaCl/100 gm of water of sodium chloride (NaCl) and 2mg of potassium meta-bisulphate/1000gm of water for 10 minutes and followed by preparation of mash in a mixer grinder. The potato mash was gelatinized in an autoclave at 10 psig pressure for 15 minutes (**Chakraborty *et al*, 2013**). Then glycerol monostearate (GMS) was weighed in different amounts (1%, 2%, and 3% respectively) then mixed with a refined vegetable oil and water in a ratio of 2:1:10 respectively and heated in boiling water bath (90-100°C) and stirred till

GMS gets evenly dispersed to form slurry. The potato mash and water was added (in a ratio of 10:1 respectively) to the three different slurries and stirred at 300 rpm for 10 minutes in a magnetic stirrer (Eltek, Model – 2011) to form a thick foam slurry.
The foam mat drying and thin layer hot air drying experiments were carried out in a batch type tray drier (Suan Scientific Instruments &Equipments). The drier was equipped with an electrical heater, blower (230rpm) and temperature indicators. It consisted of trays (800X400X30mm) with perforations of diameter 7mm and a temperature controller (0-200°C).
The tray drier was run intermittently in order to stabilize the desired temperatures (i.e. 50°C, 55°C, 60°C respectively) inside the chamber. The homogeneous foamed potato was poured to Petri plate to equal thickness of 10mm and equal weight of 10gms each and kept for drying. Same was done for non foamed potato mash. The trays were then placed on the tray stand in position for drying. The foamed and non-foamed potato slurries were dried at different temperatures until constant weight. The Petri plates were taken out of the drying chamber at different time intervals for determination of weight loss. The loss in weight was recorded using Dhona balance having least count of 0.1 mg on initial and final weight basis. Final moisture content of each of the sample was obtained by A.O.A.C method. A crispy powder was obtained which was grounded to a fine powder and packed in LDPE zip pouches (0.06mm film thickness) separately.

Determination of Thermophysical Properties

The thermophysical properties i.e. specific heat, density, thermal conductivity and thermal diffusivity of the thin layer potato mash and the foamed potato mash were calculated from Choi and Okos model (**Choi and Okos, 1986**). The compositions of the potato mashes were determined at different time intervals with the different sample weights obtained and the mass fractions and volumetric mass fractions were calculated from it.

Prediction of Specific Heat

The specific heat of a food is defined as the quantity of thermal energy associated with unit mass of the food and a unit of change in temperature. A general prediction model for analysis of specific heat of food as a function of its composition and drying temperature has been developed by Choi and Okos (**Choi and Okos, 1986**) based on an extensive study and analysis of specific heat data for many types of food with different compositions and over a temperature range of 20-100°C (**Heldman *et al*, 2002**). The specific heat of the total product is expressed as a summation of the product of component specific heat and mass fraction of the component:

$$c_p = \Sigma(c_{pi} M_i) \quad (1)$$

where c_p is the total specific heat, c_{pi} is the component specific heat and M_i is the mass fraction of the product.

Prediction of Density

The general model for prediction of density was proposed by Choi and Okos (**Choi and Okos, 1986**) and involves around the product composition (M_i) and the density (ρ_i) for each component (**Heldman *et al.*, 2002**).

$$\rho = 1/\Sigma(M_i/\rho_i) \quad (2)$$

The proposed model predicts the density of high moisture food (> 60%) from the compositional information and the density relationships from the general model. For intermediate and low moisture foods (<60%) the particle density can be predicted by

$$\rho_p = e_s(\rho_s - \rho_a) + \rho_a \quad (3)$$

based on density of the product solids (ρ_s) predicted from the general model, the volume fraction of solids (e_s) from the compositional components and the density (ρ_a) of air. The total density of the food can be calculated in a similar manner as that of the high moisture foods (**Heldman *et al.*, 2002**).

Prediction of Thermal Conductivity

The general model for prediction is based on the observations made by **Choi and Okos (1986)**. The thermal conductivity (k) of the food material can be given as

$$k = \Sigma(k_i E_i) \quad (4)$$

where the volume fraction (E_i) is estimated for each component by

$$E_i = (M_i/\rho_i)/\Sigma(M_i/\rho_i) \quad (5)$$

Since the general model does not emphasize on the physical structure of the food material so it is applicable for high moisture (>60%) foods. In cases where the discontinuous component is same or higher in concentration than the continuous component the thermal conductivity can be can be expressed as derived and proposed by **Kopelman (1966)**

$$k = k_c[\{1- E_d(1-k_d/k_c)\}/\{1-E_d^2(1-k_d/k_c)(1-E_d)\}] \quad (6)$$

where E_d is the volume fraction of discontinuous product component, k_c and k_d are the respective thermal conductivities of the moisture and solid fractions of the product derived from the general model (**Heldman*et al.*, 2002**).

Prediction of Thermal Diffusivity

The combination of these three properties is thermal diffusivity, a key property in the analysis of unsteady state heat transfer (**Heldman *et al.*, 2002**). Mathematically it is expressed as

$$\text{Thermal Diffusivity} = k/(c_p * \rho) \quad (7)$$

Statistical analysis

A main effects analysis of variance (ANOVA) was used to establish the significance of differences among the values of drying rate, specific heat, density, and thermal conductivity at the 0.05 significance level. Statistical analyses were performed using Statistica (version 7) (Stat Soft, Inc., USA).

Color Measurement of Dried Potato Powder

Color is the most important physical attribute for acceptability and determination of quality. The color of the different potato samples were estimated using Hunter Lab Colorimeter (Color flex, 45/0 spec photometer). The color was measured in terms of L*, a* and b* coordinates, where L* is the lightness (0 = black, 100 = white), a* for the red (positive values) to the green (negative values) and b* indicates the yellowness (positive values) and blueness (negative values). Total color difference (ΔE), polar coordinate chroma or saturation (C*) and L* values were used as an index to report the color quality and are calculated from the following equations:

$$C^* = (a^{*2} + b^{*2})^{0.5} \quad (8)$$

$$\Delta E = [(L^* - L_{standard})^2 + (a^* - a_{standard})^2 + (b^* - b_{standard})^2]^{0.5} \quad (9)$$

Regression equation Modeling

RSM Modeling

Relationships between the independent variables (temperature, percentage of GMS) and dependent variables (total color difference ΔE) for foam mat drying were studied. The regression equation was determined (using Statistica, version 7, Stat Soft, Inc., USA) using multiple regression technique by fitting second order regression equation (**Khuri and Cornell, 1987**) of the following type

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j + e \quad (10)$$

where β_0, β_i, β_{ii}, β_{ij} are regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively, X_i, X_j are the independent variables, Y is the dependent variables nis number of independent variables. The relationships between the responses were judged by correlation multiple R, multiple R^2 which indicates the value of correlation coefficient and co-efficient of determination between the experimental and predicted data. The significance or P-value was decided at a probability level of 0.05.

Simple Regression

A simple regression in between dependent variable (total color difference (ΔE)) and independent variable (temperature) for thin layer drying has been determined (using Statistica, version 7, Stat Soft, Inc., USA) using the first order regression equation of following type

$$Y = a + bX \quad (11)$$

Where, a, and b is the intercept and slope respectively, and Y is dependent variables and X is independent variables. The relationships between the responses were judged by correlation multiple R, multiple R^2 which indicates the value of correlation coefficient and co-efficient of determination between the experimental and predicted data. The significance or P-value was decided at a probability level of 0.05.

RESULTS & DISCUSSIONS

Determination of Thermophysical Properties

Determination of Specific Heat

Figure1 shows the change in the specific heat of potato mash samples during drying within the range of 3.9529 kJ/kg°C to 1.5579 kJ/kg°C for foamed potato mats and from 3.6890 kJ/kg°C to 1.7819 kJ/kg°C for unfoamed thin layer potato mats. During drying with decrease in moisture content the specific heat decreased accordingly. This co-relation between specific heat with moisture content correlates with work done by **Nathakaranakule *et al.* (1998)** for cashew nuts, **Chandrasekar *et al.* (1999)** for coffee, **Bart-Plange *et al.* (2012)** for maize cow pea and cashew kernel and Isa ***et al.* (2014)** for melon. One of the reasons of change in specific heat of potato mash was may be due to its change in percentage composition during drying. So as the moisture content decreases and eventually becomes negligible, the composition of the potato mash becomes constant and hence the specific heat finally becomes constant. The ANOVA analysis of specific heat at different time of drying (Table 1) shows that the variation in GMS percentage (0% for unfoamed slurry and 1% to 3% for foamed slurry) has a significant effect (at $p<0.05$ level) on specific heat before specific heat become constant. Whereas, variation temperature of drying has a significant effect on specific heat only at the initial stages (Table 1). One probable cause is initially when the temperature was rising up it had a significant effect on specific heat. But as temperature elevation stopped no further significant effect on specific heat was observed.

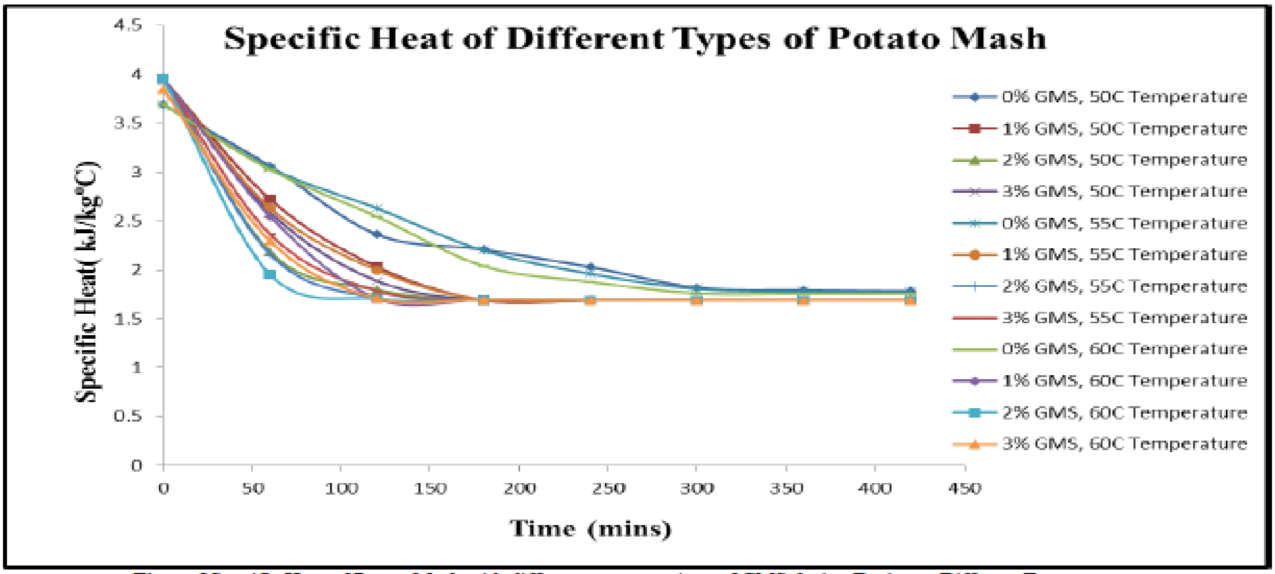

Figure 1 Specific Heat of Potato Mash with different concentrations of GMS during Drying at Different Temperatures

Table 1 ANOVA analysis of specific Heat (C_p)

	Variation of percentage of GMS		Variation of drying temperature	
Effect	F value	P value	F value	P value
60 min C_p	100.69	0.00002*	7.2629	0.02498*
120 min C_p	25.482	0.00082*	1.0780	0.39812
180 min C_p	37.780	0.00027*	0.84826	0.47377
240 min C_p	9.1565	0.01172*	0.89873	0.45561
300 min C_p	0.20068	0.89228	0.94526	0.43968

*- significant at $p<0.05$ level

Determination of Density

Figure 2 shows the increase in density of potato mash within the range of 1024.47kg/m^3 to 1555.21kg/m^3 for foam mat drying and from 1073.66 kg/m^3 to 1522.81 kg/m^3 for thin layer drying. This change in density with decrease in moisture content was also observed by **Kibar*et al.* (2010)** for rice, **Seifi and Alimardani (2010)** for corn, **Gupta and Das (1997)** for sunflower seed and **Balasubramanian (2001)** for cashew. The ANOVA of density at different time of drying shows that a significant difference in density is observed mainly for variation in percentage of GMS (0% for unfoamed slurry and 1% to 3% for foamed slurry) at $p<0.05$ level (Table 2). The probable cause is during foaming the volume of the potato mash increased due to air incorporation. As drying progressed the moisture was removed and the volume decreased. Since the volumetric contraction was higher than moisture removed, so it may be a reason why density increased with decrease in moisture content. The variation of temperature has a significant effect on density only at 120 minutes. This might be due to that after 60 minutes the temperature elevation stopped (which discussed previously for specific heat). After that, the penetration of temperature to the core of the foamed and unfoamed slurry started and almost reached completion at 120 minutes.

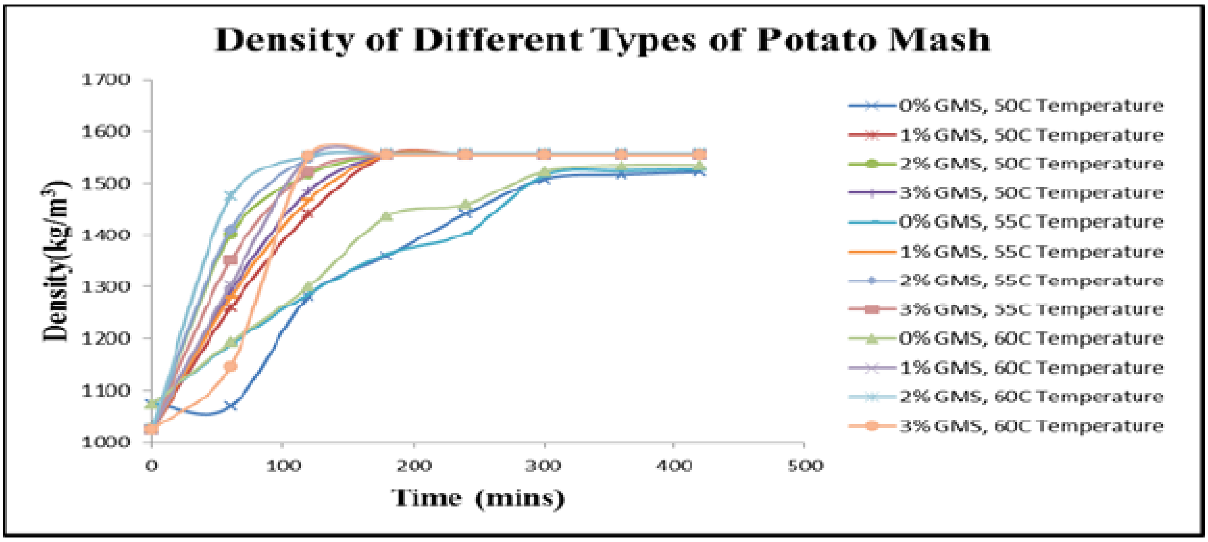

Figure 2 Density of Potato Mash with different concentrations of GMS during Drying at Different Temperatures

Table 2 ANOVA analysis of Density

	Variation of percentage of GMS		Variation of drying temperature	
Effect	F value	P value	F value	P value
60 min Density	7.6260	0.01802*	0.53722	0.61007
120 min Density	79.917	0.00003*	6.7141	0.02945*
180 min Density	46.948	0.00015*	1.0884	0.39509
240 min Density	54.850	0.00009*	1.1014	0.39136
300 min Density	99.743	0.00002*	1.4908	0.29811

*- significant at p<0.05 level

Determination of Thermal Conductivity

Figure 3 shows the decrease in thermal conductivity within the range of 0.5903 W/m°C to 0.2694 W/m°C during foam mat drying and 0.5626 W/m°C to 0.2912 W/m°C for thin layer drying. The positive relationship of thermal conductivity with moisture content of all the potato mash samples agreed with other researchers such as **Perusella*et al.*, (2010)** for banana, **Bart-Plange*et al.*, (2009)** for cowpea and maize and **Singh and Goswami (2000)** for cumin seeds. Since water has a higher thermal conductivity compared to dry agricultural materials and thus may contribute to high thermal conductivity in them at the initial stages of drying. As drying progressed the thermal energy decreased which may be as a result of removal of large amount of moisture from it. The ANOVA analysis (Table 3) shows that throughout the process a significant difference was observed mainly for variation in percentage of GMS (0% for unfoamed slurry and 1% to 3% for foamed slurry). The addition of GMS incorporates the air into the potato mash. Due to variation of air incorporation for different percentage GMS initial volume was different for different potato slurry. Hence, the variation of GMS has a significant effect on thermal conductivity at p<0.05 level.

Table 3 ANOVA analysis of Thermal Conductivity

	Variation of percentage of GMS		Variation of drying temperature	
Effect	F value	P value	F value	P value
60 min Thermal conductivity	31.457	0.00046*	0.94329	0.44034
120 min Thermal conductivity	35.194	0.00033*	1.2146	0.36067
180 min Thermal conductivity	243.62	0.00000*	3.1447	0.11638
240 min Thermal conductivity	24.663	0.00090*	1.4600	0.30434
300 min Thermal conductivity	14.973	0.00342*	3.2019	0.11318

*- significant at p<0.05 level

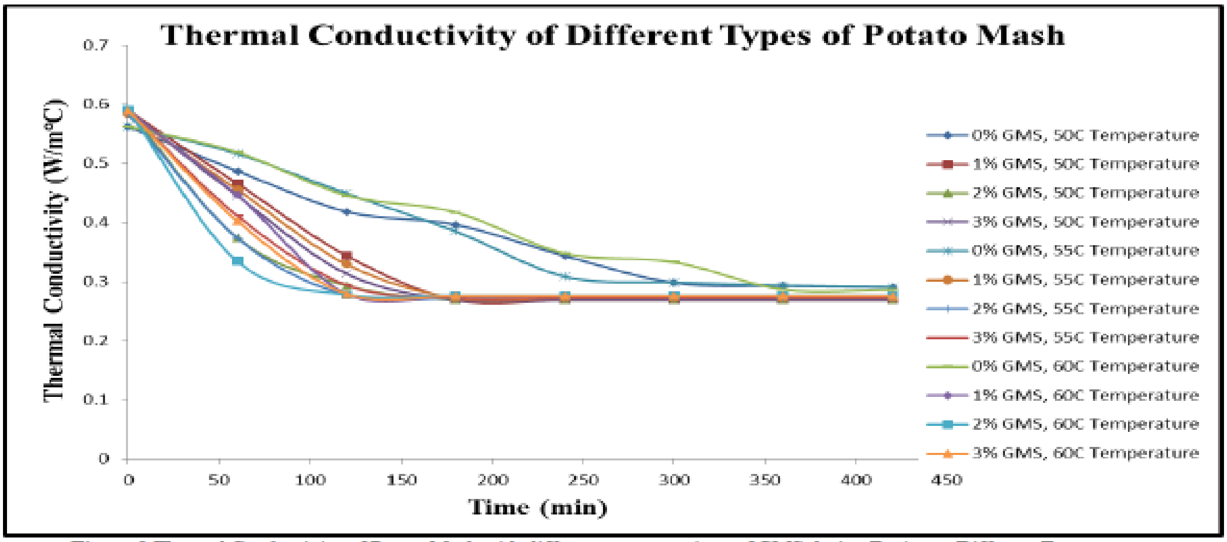

Figure 3 Thermal Conductivity of Potato Mash with different concentrations of GMS during Drying at Different Temperatures

Determination of Thermal Diffusivity

Figure 4 shows the decrease in thermal diffusivity within the range of 1.4644 X 10^{-04} m^2/s to 1.0271 X 10^{-04} m^2/s for foam mat drying and from 1.4372 X 10^{-04} m^2/s to 1.0717 X 10^{-04} m^2/s for thin layer drying. **Hobani and Al-Askar, (2000)**, found the thermal diffusivity of Khudary and Sufri dates to decrease linearly with decreasing moisture content. Other researchers such as **Aviara and Haque (2001)**, **Tansakul and Lumyong (2008)**, **Shyamal et al., (1994)** reported a positive relationship between thermal diffusivity and moisture content for sheanut kernel, straw mushroom and wheat respectively. Moisture has a higher ability to conduct thermal energy than dry materials. Since the potato mashes contained high percentages of liquid the initial thermal diffusivity was high. As time elapsed the removal of moisture took place which may be the cause of decrease in thermal diffusivity and hence when the moisture content became negligible the thermal diffusivity also became constant.

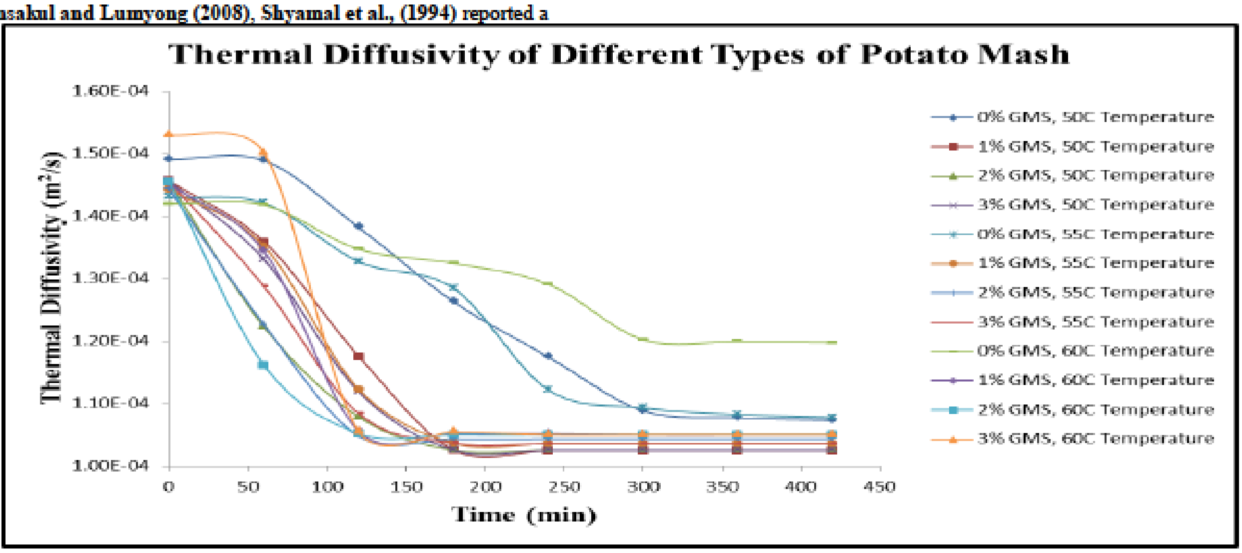

Figure 4 Change in Thermal Diffusivity of Potato Mash with different concentrations of GMS during Drying at Different Temperatures

Color Measurement of Dried Potato Powder

It was observed that L* values are higher in case of foam-mat drying than in case of thin layer drying and the values increased as the drying air temperatures increased from 50 to 60°C in all the cases (Table 4). This may be due to the fact that foam mat drying with addition of GMS up to 2% decreased the drying time by almost 50% so the length of exposure to heat was less and thus the whiteness of the powder was preserved but above 2% GMS there was no significant change. **Shi *et al.* (1999)** for tomato and **Jakubczyk *et al.* (2011)** for foam-mat dried apple showed that higher L* value is desirable in dried products. The total color difference (ΔE) between raw potato and foam mat dried potato was less than that between raw and thin layer dried potato. The color saturation or vividness (C*) of the foam mat dried powder is comparable to the fresh raw potato slices. Drying at 60°C with 2% GMS has shown the saturation of 16.44 which is closest to the standard raw potato.

Table 4Effect of Drying on Color of Potato Powder

Temperature (°C)	%GMS	L*	a*	b*	ΔE	C*
Raw Potato Slices (Standard)		69.33	-1.47	15.71	-	±15.78
50	0	65.75	2.01	23.35	±9.126	±23.43
	1	71.85	1.97	21.29	±7.023	±21.38
	2	72.79	1.83	20.48	±6.754	±20.56
	3	72.87	1.85	21.10	±7.253	±21.18
55	0	63.37	2.20	23.83	±10.723	±23.93
	1	73.39	1.68	18.35	±5.777	±18.42
	2	74.77	1.32	17.18	±6.288	±17.23
	3	74.79	1.37	17.91	±6.535	±17.96
60	0	61.53	2.45	24.05	±12.073	±24.17
	1	75.94	1.01	19.32	±7.929	±19.34
	2	78.70	0.89	16.42	±9.688	±16.44
	3	78.63	0.93	17.38	±9.748	±17.40

Regression Analysis

RSM Modeling

The RSM modeling was fitted to response data of total color difference (ΔE). It was found that temperature and square term of temperature both have significant effect on total color difference (ΔE) at $p<0.05$. The model equation is

$$T.C.D\ (\Delta E) = 227.4916-8.1574*T+0.0746*T^2-3.1052*\%GMS-1.992*\%GMS^2+0.0795*T*\%GMS \quad (12)$$

In equation 12, T denotes temperature (°C). The value multiple R (0.98) and multiple R^2 (0.97) was found to be very good which indicates that the model was very effective for prediction of total color difference.

Simple Regression

A simple regression has been done to obtain the model equation for dependent variable (total color difference (ΔE)) and independent variable (temperature) for thin layer drying (Table 4). The model equation is

$$T.C.D\ (\Delta E) = -5.56783+0.2947*Temperature\ (^0C) \quad (13)$$

It was found that temperature has significant effect on total color difference (ΔE) at $p<0.05$. The value multiple R (0.99) and multiple R^2 (0.99) was found to be very good which indicates that the model was very effective for prediction of total color difference.

Joglekar and May (1987) have suggested for a good fit of a model, regression coefficient (R^2) should be at least 80%. The above two model (i.e. equations 12, 13) can be effective for prediction purpose because R^2 value is more than 80% (i.e. $R^2 > 0.80$).

CONCLUSION

Change in temperature and degree of foaming agent significantly affected the thermal properties of potato. As drying progressed the decrease in moisture content showed a considerable decrease in specific heat capacity, thermal conductivity and thermal diffusivity while density increased due to loss of moisture. Knowledge of these thermal properties is important for mathematical modelling and simulation of heat and moisture transport systems. However, it is also observed that the moisture content significantly affects different thermal characteristics.The variations in temperature also had significant effect on the color of the potato. Moreover the dried sample with 2% GMS seemed to have retained the color comparable to the original color of raw potato.

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JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

A PRELIMINARY STUDY ABOUT GLUTEN LEVELS IN SARDINIAN CRAFT BEERS

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doi: 10.15414/jmbfs.2017.6.5.1195-1198

ARTICLE INFO

Received 1. 9. 2016
Revised 2. 2. 2017
Accepted 21. 2. 2017
Published 3. 4. 2017

Short communication

OPEN ACCESS

ABSTRACT

The aim of this study was to determine the gluten content of a representative sampling of Sardinian craft beers. Twenty-five craft beers produced by seven Sardinian microbreweries were analyzed. All beers were produced without micro-filtration, pasteurization or preservative additions provided by Italian law. The competitive enzyme immunoassay (Ridascreen competitive ELISA kit) was used for gluten quantification. The levels of gluten found ranged from 39 mg/L in a Pilsner beer to 2400 mg/L in a Weizen. No gluten-free beer was found and eight (32%) of the twenty-five beers analyzed contained less than 100 mg/L of gluten and could be labeled as "very low gluten" according to the Commission Regulation (EC) No 41/2009.
Furthermore a significant positive correlation between gluten content and foam stability was found.

Keywords: Craft beer; Beer; ELISA; Antibody R5; Celiac disease; Gluten-free beer; Foam stability

INTRODUCTION

It is well known that beer is one of the most consumed alcoholic beverages all over the world. Its average annual consumption is 74 and 86 liters per capita in Europe and Northern America respectively (**Hager *et al.*, 2014**). In Italy it is around 29.2 liters per capita whereas in Sardinia, an Italian region in the center of the Mediterranean Sea, it is almost double (**Assobirra, 2011**).
Currently, beer consumption is appreciably shifting towards the less explored world of craft beer (**Assobirra, 2015**). Craft beer is mainly chosen according to different flavor preferences and is mainly drunk by habitual beer drinkers in places traditionally connected to its consumption such as pubs and restaurants (**Aquilani *et al.*, 2015**). It is perceived to be of higher quality than industrial beer. Moreover, its antioxidant capacity is higher (**Sanna and Pretti, 2015; Tubaro, 2009**).
As sanctioned by the Italian law(**Legge 28 luglio 2016, n. 154, 2016**), craft beer must be produced by microbreweries, small and independent companies which produce limited amounts of beer (less than 200.000 hectoliters per year) and must be neither micro-filtered nor pasteurized. Furthermore craft beers are often bottled with yeast and sugar to naturally produce carbon dioxide within the bottle in a process called bottle refermentation (**Briggs, 2004**).
The experience of microbreweries in Sardinia started quite early, if compared with the rest of Italy. It was in 1993 when the micro-brewery Adis Scopel (Guspini, Sardinia, Italy) proposed its beers to the local market. In 2016 twenty-two microbreweries in operation could be counted in the region, evenly distributed throughout a territory that has a little more than 1.6 million inhabitants.
Since for brewing usually gluten-containing barley malts are used, some amount of gluten is expected in the final product and it may be toxic for celiac patients. Celiac Disease (CD) is an autoimmune disorder characterized by a chronic inflammation of the small intestine due to a permanent intolerance to gluten proteins (or to their fragments produced during digestion) found in wheat, barley and rye (**Selimoglu*et al.*, 2010**). Gluten proteins are composed of prolamins and glutelins located in the starchy endosperm of the cereal. The most troublesome components of gluten are the prolamins; such as gliadin from wheat, hordein from barley, secalin from rye and avenin from oats (**Van Landschoot, 2011**). The functional changes in celiac patients include reduction in food digestion, decreased absorption of macro and micronutrients and increased secretion of water and solutes (**Rostom*et al.*, 2006**).
Combining the cases of NCGS (Non-Celiac Gluten Sensitivity) with those of CD, the incidence rises alarmingly to 6 people out of 100 worldwide (**Genetics home reference, 2015**). According to the Annual Report of the Italian Ministry of Health, CD prevalence in Italy is 0.28%, but it is thought to be underestimated (Figure 1). Sardinia is the Italian region with the highest prevalence of CD (**Italian Ministry of Health, 2014**).
CD seems to be highly correlated with other autoimmune diseases (**Gujral et al., 2012**), so its relatively high rate in Sardinian population is not surprising. The Mediterranean island of Sardinia is in fact well known for being a genetic isolate with a particularly high prevalence of autoimmune diseases (**Sardu et al., 2012**). As observed all over the world, CD prevalence is also increasing in Sardinia (Figure 1) with the total number of diagnosed CD patients passing from 3169 in 2007 to 6145 in 2014 (**Italian Ministry of Health, 2008, 2015**). For these reasons, gluten content characterization of food and beverage appears impellent.

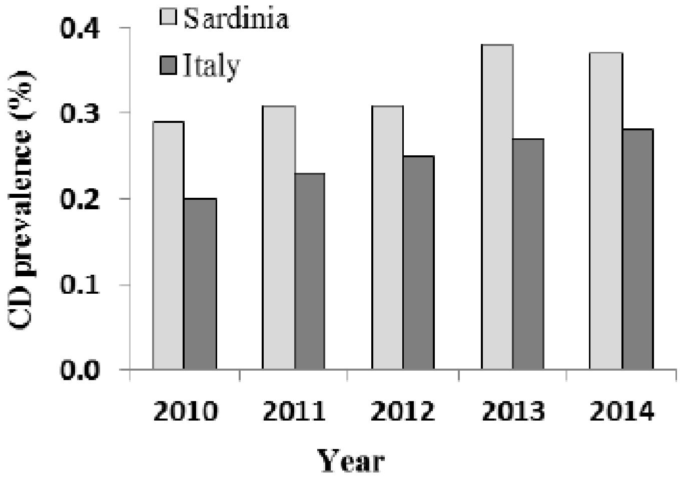

Figure 1 Increasing of Celiac Desease (CD) prevalence in Italy and Sardinia from 2010 to 2014. Data from the annual reports on Celiac Disease of the Italian Ministry of Health (2011, 2013, 2014, 2015).

Elimination of sources of gluten from the diet is the only effective remedy to lead a normal life. Therefore, it is very important to know the effective daily dose of gluten intake, without neglecting the hydrolyzed forms present in fermented products such as beer, and a correct characterization and proper labeling of products becomes essential.

In 2009 the Codex Alimentarius Commission asserted that gluten-free foods cannot contain wheat, rye, barley, oats or their crossbred varieties, unless they have been specially processed to reduce the gluten level below 20 ppm (**Codex Alimentarius Commission, 2008**).
Several commercial test kits for gluten quantification are available and the majority are based on ELISA (enzyme linked immunosorbent assay). The official standard method for gluten determination according to the Codex Alimentarius is an ELISA which uses the R5 antibody. Nowadays, second generation competitive ELISA based on the Mendez R5 antibody is considered the most reliable method to detect partially hydrolyzed prolamins in beer (**Panda *et al.*, 2015; Hager *et al.*, 2014; Haas-Lauterbach*et al.*, 2013**).
Considering the massive amount of beer consumed in Sardinia, the number of craft breweries and the increase of diagnosed CD patients on the island, we made a screening of the most representative craft beer styles produced in Sardinia to quantify their actual gluten content. In order to assess the productive range of the island, eighteen different beer styles were selected with an alcohol content in the range from 3.59 %V/V to 8.15% V/V and different grist composition.

MATERIAL AND METHODS

Beer sampling

Twenty-five bottled beers produced in Sardinia by seven local micro-breweries were analyzed: seventeen all-barley malt (one Altbier, two Amber Ale, one America IPA, one Bitter, one Dubbel, one Golden Ale, one Helles, one Imperial Stout, one Italian Grape Ale, two Pale Ale, three Pilsner, one Rauchbier and one Strong Ale) and eight wheat content beer (three Blanche, one Saison, one Stout, two Weizen and one White IPA). For gluten determination and beer analysis three bottles of each brand and beer style were analyzed.

Determination of gluten contents

Analysis of gluten content in beer was performed by a competitive immune enzymatic method because it is the method of choice for the determination of peptide fragments in beers and other food products containing hydrolyzed proteins (**Codex Alimentarius Commission, 2008**).Analysis was carried out by the RIDASCREEN Gliadin competitive assay (Art. No. R7021, R-Biopharm, Darmstadt, Germany) according to the manufacturer's instruction. The monoclonal antibody R5 recognizes several small repetitive celiac-toxic epitopes (QQPFP, LQPFP, QLPYP, QLPTP, QQSFP, QQTFP, PQPFP, QQPYP and PQPFP). Since the epitope QQPFP is present in wheat gliadin, barley hordein and rye secalin, antibody R5 recognizes fractions in all of these grains (**Haas-Lauterbach*et al.*, 2012**).
Bottles were shaked for 3 minutes in order to suspend the pellet on the bottom and 100 ml of beer was poured in a beaker and degassed; 1 ml sample was used for gluten determination. Gliadin concentrations were converted into gluten concentrations by multiplying the test results by a factor of 2 (**Codex Alimentarius Commission, 2008**). The beer containing wheat was diluted to a final dilution factor of 2500; the all-barley malt beers were diluted to a final dilution factor of 500 as described in the assay. A commercially available gluten-free beer made from barley malt (Peroni gluten-free, Peroni, Roma, Italia) was used as negative control, and the kit prolamine-free solution was used as blank.

Beer analysis

The original extract (% w/w), apparent extract (% w/w) and alcohol content (% V/V) were measured with a DMA 4500 density meter (model PBA-B Generation M, Anton Paar, Graz, Austria). Foam stability was measured with a NIBEM-TPH foam stability tester (Haffmans, Zeist, The Netherlands) according to the official Analytica EBC method 9.42.1 (**European Brewery Convention, 1998**). Analysis was conducted at 20°C, using carbon dioxide to generate a standard glass full of foam. The Nibem tester measures the foam collapse time over a distance of 30mm. Turbidity analysis was performed according to the official Analytica-EBC method 9.29 (**European Brewery Convention, 1998**) using a Turbidity meter (model HI-93703 Hanna Instruments) and was expressed in EBC units.

RESULTS AND DISCUSSION

Determination of gluten contents in Sardinian craft beer samples

Gluten level of industrial beers has been analyzed by competitive ELISA in precedent studies (**Guerdrum and Bamforth, 2011; Van Landschoot, 2011; Dostálek*et al.*, 2006**), but gluten content of Sardinian craft beers was here analyzed for the first time. Table 1 shows the measured gluten levels and data analysis of twenty-five craft beers.

Table 1 Gluten levels, original extract, apparent extract, alcohol content, turbidity and foam stability in craft beers analyzed

Beer style	Gluten (mg/L)	Original extract (% w/w)	Apparent extract (% w/w)	Alcohol content (% v/v)	Turbidity (EBC units)	Foam stability (s/3 cm)
Altbier *	163±19	14.08±0.02	3.13±0.01	5.91±0.01	1.4±0.1	286±12
Amber ale *	97±1	17.16±0.24	3.92±0.06	7.30±0.09	2.0±0.4	242±2
Amber ale *	90±0	11.78±0.02	3.29±0.01	4.52±0.01	2.4±0.2	251±5
IPA *	153±6	15.90±0.01	3.32±0.01	6.87±0.01	1.2±0.5	269±31
Bitter *	61±1	11.42±0.03	3.12±0.01	4.43±0.01	11.5±0.2	265±4
Blanche #	162±8	15.44±0.22	4.32±0.02	5.93±0.12	6.6±0.5	270±8
Blanche #	459±120	12.52±0.01	3.21±0.01	4.97±0.01	1.2±0.2	203±1
Blanche #	490±14	12.01±0.03	2.88±0.02	4.85±0.04	10.3±0.1	282±15
Dubbel *	177±47	18.46±0.16	6.70±0.01	6.59±0.06	24.6±0.6	286±9
Golden ale *	53±18	12.28±0.01	2.59±0.01	5.16±0.01	3.1±0.3	251±1
Hells *	124±43	12.44±0.02	3.14±0.01	4.96±0.01	22.6±1.1	217±14
IGA *	227±49	20.18±0.01	5.74±0.01	8.15±0.04	2.3±0.6	268±7
Pale ale *	112±19	13.07±0.17	3.00±0.05	5.37±0.11	61±2.5	244±5
Pale ale *	198±0	12.87±0.08	2.68±0.02	5.44±0.04	2.0±0.2	286±8
Pilsner *	108±10	12.33±0.01	2.75±0.01	5.06±0.01	1.8±0.2	231±11
Pilsner *	50±7.0	11.30±0.04	2.38±0.02	4.73±0.02	2.1±0.6	204±6
Pilsner *	39±7	13.13±0.02	3.36±0.02	5.24±0.01	2.1±0.5	217±10
Rauchbier *	80±6	13.02±0.01	3.65±0.01	5.00±0.03	4.8±0.9	236±15
Saison #	170±3	14.09±0.03	3.48±0.01	5.74±0.01	5.4±0.1	289±1
Stout *	182±38	17.11±0.01	4.45±0.01	6.94±0.01	5.3±0.2	299±16
Stout #	153±13	11.16±0.01	4.62±0.01	3.59±0.01	55.1±0.1	168±3
Strong ale *	49±3	17.53±0.03	4.06±0.01	7.42±0.02	1.8±0.3	236±1
Weizen#	500±0	12.27±0.01	2.13±0.01	5.40±0.01	4.3±0.1	250±4
Weizen#	2400±437	12.56±0.01	2.65±0.01	5.29±0.01	3.6±0.6	164±12
White IPA #	224±22	11.22±0.02	2.32±0.02	4.71±0.02	1.6±0.1	289±7
Gluten free	<10**	10.55±0.01	1.52±0.01	5.29±0.01	1.1±0.1	176±8

Values are averages of three measurements ± the standard deviation. *All barley-malt beers. #Wheat or wheat malt in grist composition. ** Results beyond the quantification limit of the ELISA used

As expected, beer including wheat or wheat malt in the total grist, contained higher levels of gluten (from 150 to 2400 ppm) compared with beers without wheat or malted wheat in the grist (39 to 220 ppm). None of the craft beers analyzed was gluten-free. The lowest level found was 39 mg/L in a Pilsner beer and the highest was 2400 mg/L in a Weizen. In eight beer samples (32%) we found a level of gluten below 100 mg/L. These beers could be labeled as "very low gluten" according to the Codex Alimentarius Commission Standard (**Codex Alimentarius Commission, 2008**). In a previous study (**Guerdrum and Bamforth, 2011**), a range of commercially available industrial beers was analyzed using the same Ridascreen Gliadin Competitive ELISA kit. 36% of those beers turned out to be gluten-free and all of them were "low in gluten" with the exception of a wheat beer. As reported above, unlike industrial beers, the craft beers analyzed were produced without a stabilization process, pasteurization or micro-filtration and they were bottle-fermented. Besides, industrial beers are often stabilized ~~beer~~ before packaging by kieselguhr filtration, polyvinylpolypyrrolidone filtration (PVPP) or silica gel. Large-scale breweries often follow a general formula and maize or rice products are often added to cut beer manufacture costs (**Briggs, 2004**). Maize and rice are gluten-free ingredients and contribute to keep low gluten level in finished beer. Stabilization processes and different grist composition may result in relatively low gliadin levels in industrial beers.

Gluten content relationship with foam retention, turbidity and original extract

A correlation analysis among gluten content and all-barley malt beer analysisis shown in Figure 2. Gluten composition is well known for having influence on chemical-physical characteristics of beer foam (**Bamforth and Kanauchi, 2003; Steiner *et al.*, 2010**). Moreover different authors reported evidence that some hordein species improve quality and persistence of beer foam (**Sheehan and Skerritt, 1997; Vaag*et al.*, 1999, Evans *et al.*, 2003**). The significant correlation between gluten content and foam stability found in this study (Figure 2a) was not surprising. As already described by Bamforth and Kanauchi (2003) this may be due to the interaction between hop acids and some members of the hordein storage protein family deriving from malt.

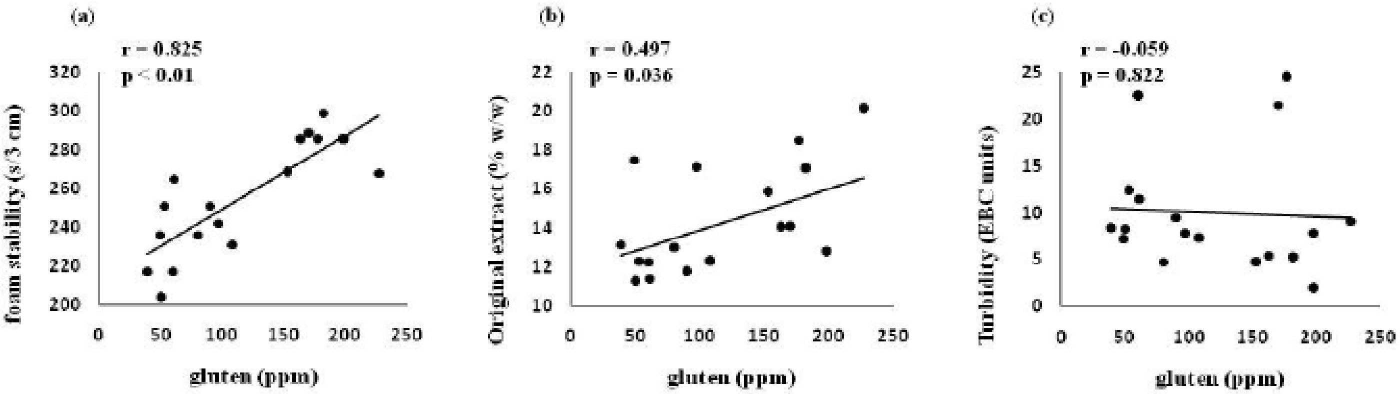

Figure 2 Relationships between gluten content and foam stability (a), original extract (b) and turbidity (c) among all-barley malt beers. Correlation was performed using Pearson correlation coefficient.

According to **Steiner *et al.*, (2010)**, the same hordein storage proteins are also responsible for turbidity in beer. Nonetheless, we found no correlation between the two (Figure 2c). This could be explained by interaction of several variables more frequently occurring in craft beers than in industrial beer such as presence of yeasts (deriving from the bottle fermentation). Furthermore yeasts flocculation ability (the ability of yeasts to settle on the bottom at the end of refermentation) and cell number, can vary from beer to beer and cause turbidity independently from gluten content (**Boulton, 2001**).

It appears more difficult to explicate the lack of correlation (Figure 2b) that was found between gluten content and original extract (the amount of sugar in wort before fermentation) which directly derives from a higher amount of malt used to produce beer and therefore more gluten potential. A possible explanation could be the high variability observed in craft beer production, both among microbreweries and within the same plant, due to the lack of automation in the production process which makes it entirely dependent on manpower skills.

CONCLUSION

Although literature shows a relative low gluten content for commercial beers, with a high percentage of unaware gluten-free beers, a surprising high gluten level was found in Sardinian craft beers. Even if 37% of the analyzed beers could be labeled as "very low gluten", we found no gluten-free beers among our samples. Considering the high rate of gluten intolerance in Sardinia population,and the high etherogenity level of individual sensitivity, gluten sensitive persons should be careful when drinking craft beer. Moreover, correlation analysis highlight the relevance of gluten content on foam stability of craft beers.

Acknowledgments: The authors would like to thank Marduk Brewery, P3 brewing Company, Barley Brewery, Nora Brewery, Domusnovas Brewery and Birrificio di Calgliari for beer supplies.

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JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

DEVELOPMENT OF OYSTER MUSHROOM POWDER AND ITS EFFECTS ON PHYSICOCHEMICAL AND RHEOLOGICAL PROPERTIES OF BAKERY PRODUCTS

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doi: 10.15414/jmbfs.2017.6.5.1221-1227

ARTICLE INFO

Received 11. 1. 2017
Revised 24. 2. 2017
Accepted 15. 3. 2017
Published 3. 4. 2017

Regular article

OPEN ACCESS

ABSTRACT

Wheat flour is staple diet and is mainly consumed in the form of chapatti and bread in Pakistan. Wheat is deficient in lysine which is the first limiting amino acid in wheat. Proteins are indispensable constituents of human diet and are considered crucial for the growth, maintenance and repair of the body tissues. They are one of the essential building blocks of all life. The objective of this study was to prepare the composite flour by blending straight grade flour with oyster mushroom powder (OMP) in different proportions for the baking of bread. The flour was also evaluated for rheological properties. The supplemented bread was subjected to sensory evaluation and physical analysis by Farinograph measurements of the following characteristics affected by mushroom powder: water absorption, dough development time, softening of dough, dough stability and mixing tolerance index. Results showed that OMP increased water absorption capacity of dough with highest absorption recorded in T_5 (66.4%) and minimum in T_0 (56.4%). Dough development time increased with the increasing amount of OMP. It was minimum in T_0 (1.5 min) and maximum in T_5 (4.6 min). The capacity of dough to get soft also increased with increasing treatment level. Dough stability also decreased with increasing level of OMP. Mixing tolerance index was also significantly affected by OMP. Results of amylograph indicated that peak viscosity increased from 1595 to 1700 BU from T_1 (3% mushroom powder) to T_5 (15% mushroom powder) with the increase of OMP.

Keywords: Amylograph, Bakery Products, Farinograph, Mushroom Powder, Protein, Bread

INTRODUCTION

In many cultures, edible mushrooms have been consumed as food and medicine for many years (**Wan Rosil *et al.*, 2011; Akbarirad *et al.*, 2013**). They constitute a significant food item regarding health, human nutrition and disease prevention (**Chang and Miles, 2004**). It is generally said that "foods and medicines have a common origin" (**Kaul, 2001**). Mushrooms are versatile food items which may be consumed fresh or cooked wholly. Mushrooms have been included in a normal human, diet for so long but currently, amounts consumed have increased to a great extent involving a larger numbers of species. Nowadays, mushrooms are eaten for their distinctive flavor, texture as well as for the health benefits which they provide (**Chang and Miles, 2004**).

Mushrooms have a high nutritional value, being fairly well off in protein, having the appropriate amount of essential amino acids and fiber but with low fat content. Considerable amounts of vitamins (C, D, E, B_1, B_2, and B_{12}) are provided by edible mushrooms (**Mattila *et al.*, 2001**). The edible mushroom can be the best food for diabetic patients and for those who desire to get rid of excess fat since it contains small quantities of carbohydrate and fat (**Deepalakshmi and Mirunalini, 2014**). In the developing countries, oyster mushrooms considerably contribute in overcoming protein deficiency. Also, they are rich in calcium, iron, potassium, copper, zinc, and manganese (**Owaid, 2013**). Particularly, to produce mushroom capsule and extract special strains of dried mushrooms are used. The mushroom is a rich food and in addition to being a satisfying meal, it is unrivaled for flavor (**Alam and Raza, 2001**). Dietary mushrooms offer a broad range of curative properties. Oyster mushroom has important therapeutic properties including antibacterial, antifungal, antiviral, anticancer activities, blood lipid lowering effects and immunity. Mushrooms have also been considered helpful against insomnia, cancer, asthma, diabetes, cholesterol reduction, allergies and stress (**Wang *et al.*, 2000**).

Fresh mushrooms being perishable start deterioration instantly within a day after harvest. Because of the extremely delicate nature of fresh mushrooms, they have to be preserved. Many post-harvest procedures can be applied to improve the shelf life. Preserve the quality of mushrooms and enhance storage. This will play a fundamental role in their commercialization (**Gothandapani *et al.*, 1997**). Many methods are used for preservation of mushroom but canning is the most commonly implemented methods at a commercial scale. There are many other ways used to increase the shelf life of mushroom like drying, pickling etc. Drying is a reasonably easy and frequently used technique among several methods for the preservation of mushrooms (**Rai *et al.*, 2003**). However, drying rate is influenced by many factors which include the thickness of mushroom, moisture diffusivity, temperature and method of drying (**Yapar *et al.*, 1990**).

For the diversification of bakery products many nutritionally rich ingredients are added in them (**Sudha *et al.*, 2007**). Non-wheat flours which are rich in protein can be incorporated in different products to avert protein deficiency (**Sharma and Chauhan, 2002**). Development and consumption of such therapeutic bakery products like bread would be helpful in raising the nutritional status of the masses. In this light, bread technology probably constitutes one of the oldest technologies known to man (**Selomulyo and Zhou, 2007**). Bread is in fact an important food item considered to be one of the commonly used food products in the world. It is the staple food for many countries and is principally made of hard wheat flour, fat, yeast, salt, sugar and water (**Badifu *et al.*, 2005**). This cereal product has low protein content and as such is not a balanced diet because of its low lysine and other essential amino acids (**AGU *et al.*, 2010**). Since bread is an important food that is accepted, it can be used as a convenient food item for protein fortification thereby improving the nutritional health of the people and preventing malnutrition. The premix or composite flour technique plays a significant role in balancing the deficiency of essential nutrients in wheat (**Anjum *et al.*, 2006**). This composite flour technique is actually the process of incorporating other cereals or legumes into wheat flour. As such, locally accessible raw material is therefore used for the preparation of better quality local agricultural food products in an economical way (**SHAHZADI, 2004**). It is from this perspective that while keeping in view the nutritional and medicinal importance of mushroom, this study was carried out to improve the nutritional and functional properties of bread by supplementing wheat flour with mushroom powder. The experimentation involved development of mushroom powder, evaluation of the effect of wheat supplementation with this powder on rheological, physical and chemical properties of bread, and evaluation of organoleptic properties of bread with acceptable level of mushroom powder addition.

MATERIAL AND METHODS

Samples

The research was carried out at National Institute of Food Science and Technology, University of Agriculture, Faisalabad. Oyster mushroom was obtained from Mushroom Lab of Institute of Horticultural Sciences, University of Agriculture, Faisalabad. Remaining raw material was procured from the local market. Mushrooms were dried at a temperature of 45 °C for 2 days. Then mushrooms were ground to powder form.

Proximate analysis

Proximate analysis of wheat flour and mushroom powder were performed according to their respective methods described in AACC (2000).

Rheological analysis

Dough rheology of composite flour prepared with different levels of mushroom powder (0%, 3%, 6%, 9%, 12%, and 15%) was determined by using farinograph and amylograph following the procedure is given in AACC (2000).

Farinographic studies

Wheat flour was examined for physical dough properties by using farinograph according to the procedure outlined in AACC (2000) Method No. 54-21. The farinograms were interpreted for different characteristics such as water absorption, dough development time, dough stability, mixing tolerance index and softening of dough as described below.

Water absorption

Percentage of water required to reach the center of the curve on the 500 Brabender units (BU) lines with the maximum consistency of the dough (peak) is termed as water absorption of that flour sample. For each treatment, water absorption was recorded directly from the burette attached to the farinograph.

Dough development time

This is the time required for the curve to reach its full development or utmost consistency possessing highest peak. High peak values are associated with strong flour, which have long mixing time.

Dough stability

The dough stability was recorded as the time difference between the points where the top of the curve first intersected 500 BU line (Arrival time) and the point where the top of curve left 500 BU line (Departure time).

Mixing tolerance index

The mixing tolerance index was derived in Brabender unit (BU) as the difference from the top of the curve at the peak to top of the curve after 5 minutes from the peak.

Amylograghic studies

Amylogragh characteristics of flour are related to the heating behavior of the starch content of the flour. Alpha-amylase activity was studied for index of biochemical change in BU according to the Method Number 22-12 given in AACC (2000). The straight grade flour sample and blend (flour plus mushroom powder) were run through Brabender-visco. Amylograph equipped with 65 grams capacity bowl to determine properties of dough. A sample of 65 g of flour was combined with 450 ml of distilled water (DW) and mixed to make slurry. The slurry was stirred while being heated in the amylograph, beginning at a temperature of 30 °C and increasing at a constant rate of 1.5 °C per minute until the slurry reaches 95 °C. The amylograph recorded the resistance to stirring as a viscosity curve on graph paper. Peak viscosity was the maximum resistance of a heated flour and water slurry to mixing with pins. It was expressed in Brabender Units (BU).

Product development

Wheat flour in the bread was replaced by mushroom powder as described in the treatments (Table 1). T_0 was considered as control.

Table 1 Experimental plan

Treatments	Mushroom powder (%)
T_0	0
T_1	3
T_2	6
T_3	9
T_4	12
T_5	15

Physical analysis of bread

Loaf weight and volume

Loaf weights and volumes were measured 1 hour after removal from the oven. The loaf was weighed using an electronic balance and loaf volume was measured by rapeseed displacement method given in AACC (2000). Each loaf was put in a container and covered with rapeseeds totally fill the container. After that, the loaf was removed and the volume of rapeseed was recorded using the method of **Dubat. (2010)**. Specific volume was measured by dividing loaf weight to loaf volume.

Texture analysis

The textural study of bread was conducted by using Texture analyzer (Model TA-XT2, Stable Microsystems, Surrey, UK) with a 5kg load cell as described by **Piga *et al.* (2005)**. It is an automatic equipment having software attached which gives the measurements of the hardness and resistance of the bread to bend or snap. The Texture Expert program version 4 was used for data analysis. Texture analyzer has three-point bending rig (HDP/3BP) using five-kilogram load cell heavy duty platform (HDP/90). The rig base plate with two adjustable supports that were placed at five cm distance to support the sample. This distance was kept at same for all the samples running. The bread was placed at the central area of the supports. The resistance of the sample to bend is the distance at the point of a break, related to the fracturability of the sample. The sample that breaks at a very short distance has a high fracturability otherwise low fracturability. The display shows force in gram (g) and distance in millimeters (mm). Three loaves of bread of each treatment were analyzed for the hardness (firmness) and fracturability.

Color of bread

The bread crust and crumb color were determined by colorimeter (Neuhaus color test-II, Neotec) according to the method described by **Rocha and Morais (2003)** with some modifications. It was first calibrated with the standards (54 CTn for dark and 151 CTn for light). Then the bread sample was ground and filled in Petri plates, to get the optimum reflection of light, emerged by the photocells of the color meter; reading was noted from the display.

Sensory evaluation of bread

The prepared bread loaves were evaluated by a panel of judges (10 panelist) external properties such as crust color, volume, form symmetry, evenness of bake and internal properties like crumb color, grain, aroma, texture, and taste by method of **Land and Shepherd (1988)**.

Statistical analysis

The data obtained was analyzed statistically in order to compare the effect of different treatments on different parameters of bread. The standard statistical procedure (ANOVA) was applied according to the method described by **STEEL *et al.* (1997)**.

RESULTS AND DISCUSSION

Proximate analysis of wheat flour and mushroom powder

Proximate composition of wheat flour and mushroom powder are shown in Table 2 and Table 3 respectively. In wheat flour, moisture (12.50%), ash (0.35%), crude protein (9.45%), crude fiber (0.30%), crude fat (1.25%) and NFE (76.15%) are present. These results are in close agreement to those obtained by **Ibrahium *et***

al. (2014) who analyzed the effect of replacement of wheat flour with mushroom and sweet potato flour on nutritional composition and sensory characteristics of biscuits and observed moisture ranging from 10.2-12.5%, crude protein 8.23-12.71%, crude fat 1.2-1.8%, crude fiber 0.30-0.76% and ash 0.30-0.66%.

Table 2 Proximate analysis of wheat flour

Characteristics	% age
Moisture	12.50±0.43
Ash	0.35±0.01
Crude protein	9.45±0.03
Crude fiber	0.30±0.01
Crude fat	1.25±0.04
NFE	76.15±2.66

Table 3 Proximate analysis of mushroom powder

Characteristics	%age
Moisture	7.67±0.31
Ash	7.07±0.28
Crude protein	29.60±1.21
Crude fiber	8.33±0.34
Crude fat	1.78±0.07
NFE	45.55±1.86

On its part, mushroom powder contained moisture, ash, crude protein, crude fiber, crude fat and NFE in the range of 7.67±0.31%, 7.07±0.28%, 29.60±1.21%, 8.33±0.34%, 1.78±0.07% and 45.55±1.86% respectively. These values lie within the range mentioned by **Dickeman *et al*., (2005)**. The values of wheat flour and mushroom powder are in accordance with the values mentioned by **Bano and Rajarathnian (1988)**.

Rheological properties of composite flour

Rheological properties of dough are used as quality indicators for cereal products. There are various instruments, fundamentals and empiricals which can be used for checking the rheology of dough products. Fundamental rheometry describes the physical properties of material over a wide range of strains and rates allowing direct comparison of results obtained by various testing instruments and researchers. For better quality cereal product, it is important to have knowledge about the rheological behavior of wheat flour dough (**ASGHAR *et al*., 2009**).

Farinographic studies

The farinograph is a sensitive instrument which measures the water absorption and mixing behavior during mixing. It provides information about absorption or amount of water required for dough to reach a definite consistency and secondly a general profile of mixing behavior of dough. **Plyer (1988)** has reported that flour from strong wheat varieties had the ability to absorb and retain a large amount of water.

Water absorption

The analysis of variance for water absorption of different composite flour revealed that water absorption was significantly affected by the level of supplementation. It is obvious that water absorption ranged from 56.4 to 66.4 in different flours. The maximum water absorption (66.4%) was observed in 15% mushroom powder supplemented flour while minimum water absorption (56.4%) was observed in 100% wheat flour (control). It is reported that water absorption increases with the increase in protein content. But other factors like starch damage during milling also affect water absorption (**Asghar et al., 2007**). Studies of **Hesham *et al*. (2007)** have concluded that water absorption of flour increases with the addition of mushroom powder and legume flours. The increasing water absorption may be due to the fact that raw and germinated legume and mushroom powder contain more fiber, sugars and higher protein content, which retain more water.

Dough development time

The analysis of variance for dough development time of different composite flour revealed that the dough development time was significantly affected by the level of supplementation. The mean values of dough development time ranged from 1.5 min. to 4.5 min. in different composite flour the highest dough development time (4.5 min) was observed in the 15% mushroom powder supplemented flour, while minimum dough development time was observed in 100% wheat flour (control). The dough development time increased with increase in the level of gluten quality. Dough development time is a sign of protein quality and strength of flour (**Pyler, 1988**). The results of the current study are in line with the findings of **Rosell *et al*. (2001)** who observed that dough development time increased by adding hydrocolloids in the bread.

Softening of dough

The analysis of variance for softening of dough of different composite flour revealed that the softening of dough was significantly affected by the level of supplementation. The mean values of softening of dough ranged from 255 to 70 BU in different composite flours. In this study, maximum softening of dough was observed in 15% mushroom powder supplemented flour while the minimum softening of dough was observed in 100% wheat flour.

Dough stability

The analysis of variance for dough stability of different composite flour revealed that the dough stability was significantly affected by the level of supplementation. The mean values of dough stability ranged from 1.8 min. to 5.4 min. in different composite flours. An overall increasing trend of dough stability was observed with increase in protein content. The minimum dough stability was observed with mushroom powder supplemented flour 1.8 min while higher dough stability was observed in 100% wheat flour (control). The dough stability is actually tolerance of flour to over or under mixing. It is a primary index of flour quality and is one of the most significant determinations made by farinograph (**Pyler, 1988**). It is clear from the study of **Hesham *et al*. (2007)** that dough stability decreased as the concentration of mushroom powder increased.

Mixing tolerance index

The analysis of variance for mixing tolerance index of different composite flours revealed that the mixing tolerance index was significantly affected by the level of supplementation. The mean values of mixing tolerance index ranged from 52 to 75 BU in different composite flour. An overall increasing trend of mixing tolerance index was observed with increase in protein content. The highest mixing tolerance index (75 BU) was observed in 15% mushroom powder supplemented flour while lowest mixing tolerance index (52 BU) was observed in 100% wheat flour (control).
A study of **Mueen Ud Din (2009)** related to mixing tolerance index showed that mixing tolerance index varied from 21 to 64 BU in straight grade flours. **SUDHA *et al*. (2007)** reported that by the addition of wheat bran and rice bran blend, mixing tolerance was increased significantly. The results of this study were in accordance with the result of **Lee *et al*. 2000**. Moreover, **Oh and Kim (2002)** suggested that by the addition of green tea powder, mixing tolerance index is significantly increased.

Amylographic studies

The amylograph is related to the starch content of the flour and its behavior during heating. Amylograph checks the alpha amylase activity during gelation period of starch. In the present study, wheat flour was substituted with mushroom powder due to which amylase activity decreased. Analysis of variance related to peak viscosity shown in Table 4 showed mean values of peak viscosity of different composite flour. It is clear from the table that mushroom powder has a significant effect on wheat flour. The peak viscosity was observed and it was noted that peak viscosity increased from 820 to 1700 BU. It showed that as the level of fiber increased, peak viscosity started to decreased.

Table 4 Effect of mushroom powder on peak viscosity of different composite flour

Treatment	Peak viscosity (BU)
T_0	820^{d}
T_1	1700^{a}
T_2	1659^{ab}
T_3	1635^{bc}
T_4	1610^{bc}
T_5	1595^{c}

Legend: T_0 = Control (100% Wheat flour), T_1 = 3% mushroom powder supplementation, T_2 = 6% mushroom powder supplementation, T_3 = 9% mushroom powder supplementation, T_4 = 12% mushroom powder supplementation, T_5 = 15% mushroom powder supplementation.

Sudha *et al*. (2007) studied the effect of apple pomace as a source of dietary fiber on the rheology of cake. They concluded that amylograph studies showed decrease in viscosity of peak and cold paste viscosity from 950-730 BU and from 1760-970 BU, respectively. The studies of **Sharma *et al*. (1999)** were related to the effect of cowpea flour and wheat flour blend on rheological and baking properties. They showed that peak viscosity decreased with increase in mushroom powder level. That may be due to less swelling and gelatinization of starch due to protein molecules around them.

Analysis of Bread

Physical analysis of bread

Loaf weight and volume (Rapeseed displacement method)

The results indicated that loaf volume of bread was affected significantly by different levels of mushroom powder. It is obvious from results that loaf volume was higher for the bread prepared from the non-supplemented wheat flour as compared to the supplemented wheat flour. The mean values for loaf volume of bread prepared from the different composite flour samples are showed in Table 5. The results revealed that the maximum loaf volume (575 cm^3) was obtained from the bread produced by 100% wheat flour (control) followed by the 3% mushroom powder supplemented bread (555 cm^3) while the minimum loaf volume (450 cm^3) was found in the 15% mushroom powder supplemented bread. The decrease in loaf volume of the bread may be attributed to the reduction in the wheat structure forming proteins and a low ability of the dough to entrap air. The protein quantity, alpha amylase activity, and damaged starch might have a significant effect on bread volume and baking quality for different composite flours (**Butt *et al.*, 1997**).

Table 5 Effect of mushroom powder on physical characteristics of mushroom powder supplemented bread

Treatment	Volume (cm^3)	Weight (g)	Specific loaf volume (cm^3/g)
T_0	575.00^a	160^f	3.59^a
T_1	555.00^b	170^e	3.26^b
T_2	530.00^c	180^d	2.94^c
T_3	500.00^d	205^c	2.43^d
T_4	474.67^e	215^b	2.20^e
T_5	450.00^f	225^a	2.00^f

Also, **Vittadini and Vodovotz (2003)** reported that soy flour added bread has lower loaf volume as compared to the 100% wheat flour bread. Results regarding the weight of loaf bread are shown in Table5 .Values of loaf weight increased from 160 g to 225 g by an increase in supplementation level of mushroom powder. Specific loaf volume is measured by dividing the volume of bread loaf to the weight of bread loaf. The results revealed that specific volume is significantly affected by the addition of mushroom powder. Mean values related to specific loaf volume are shown in Table 5. According to the results, specific volume varies from 3.59 to 2.00 cm^3/g. The highest value was attained by 100 % wheat flour but bread containing 15 % mushroom powder had the lowest value of specific volume. It is clear from the studies of Okafor *et al.* (2012) that values of specific loaf volume decreased from 3.73 to 1.98 cc/g by the addition of mushroom powder.

Textural characteristics of bread

Bakery products have a characteristic shape and definite texture that is accepted by the consumers. Any significant deviation from the optimal texture characteristics of the product can be considered as a reduction in quality. Texture has a significant influence on consumer's perception of a good bread quality. The most important attributes of bread include hardness and springiness, and further parameters such as chewiness, gumminess, and cohesiveness can be taken into account as well. Hardness is defined as the force required for biting bread samples, springiness is the degree to which a sample returns to its original thickness after compression. Cohesiveness is a characteristic of mastication, gumminess depends on cohesiveness and hardness; chewiness depends on springiness and gumminess (**Meretei *et al.*, 2003**).

Bread hardness by compression test

The analysis of variance results for bread hardness prepared form different composite flours are presented in **Table 6**. The results indicate that the bread hardness varied and was highly significant among different composite flours. The results showed that bread hardness ranged from 224 to 1020 g among the various bread. The highest bread hardness (1020 g) was recorded from the bread prepared with 15% mushroom powder. The lowest hardness (224 g) was recorded with the bread produced by 100% wheat flour. Similar textural and crumb grain profiles have been stated previously by means of sensorial and instrumental studies of bread (**Shittu *et al.*, 2007**).

Penetration test for bread fracturability

The results indicate that bread fracturability varied significantly among different composite flours. The fracturability of bread increased as the level of mushroom powder increased in bread. The mean values presented in Table 6 revealed that bread fracturability ranged from 37.02 to 35.09 mm among bread of various mushroom powder supplementation.

Table 6 Effect of mushroom powder on textural characteristics of mushroom powder supplemented bread

Treatments	Bread hardness (g)	Bread fructurability (mm)
T_0	224^f	35.09^c
T_1	280^e	35.16^{bc}
T_2	440^d	35.16^{bc}
T_3	552^c	36.38^{ab}
T_4	860^b	36.74^a
T_5	1020^a	37.02^a

Color

The color of bread was determined with the help of Color meter II as described by **Rocha and Morais (2003)**. The color of the bread was determined by placing the bread under photocell. The colorimeter was calibrated by using standards (54CTn for dark and 165CTn for light). It is evident that the color value differed significantly due to differences in supplementation level of mushroom powder in wheat flour. The mean values of the color of bread prepared from composite flours given in Table 7 indicated that the bread from control (100% wheat flour) had the maximum color value (162CTn) and its value decreased gradually as the level of mushroom powder supplementation increased in wheat flour. The darker color was due to mushroom powder supplementation. The bread prepared from 15% supplementation of mushroom powder in wheat flour got the minimum color value (135CTn).

Table 7 Effect of mushroom powder supplementation on color score of bread

Treatments	Color (CTn)
T_0	162^a
T_1	152^b
T_2	142^c
T_3	141c
T_4	140^{cd}
T_5	135^d

Sensory evaluation of bread

Sensory evaluation is an important criterion for quality assessment in new product development and to meet the consumer requirements. Any new product must give satisfaction and pleasure to the consumers if it has to be a part of their eating habits. For this reason, the bread prepared from wheat flour supplemented with mushroom powder was evaluated for various sensory attributes. The sensory evaluation of bread for various attributes such as volume, color, the symmetry of form, evenness of bake, the character of crust, grain, the color of crumb, aroma, taste and texture was carried out. The product was evaluated by a panel of judges and the results are described below.

Volume

The results revealed that the scores assigned to a volume of bread were affected significantly by the level of mushroom powder supplementation. It is evident from the results which are shown in Table 8 that there was a significant decrease in assigning scores to bread as the level of mushroom powder supplementation increased in flour. The highest volume score (7.85) was gained by bread prepared from control (100%) followed by 3% mushroom powder (6.71) and 6% mushroom powder supplemented wheat flour (6). The lowest volume scores (3.20) was obtained from the bread prepared from 15% mushroom powder supplemented composite flour. The decrease in volume was observed with increase in the level of mushroom powder supplementation.
Replacement of wheat flour with non-wheat flour had a certain negative effect on bread volume. **Iqbal (2007)** also found that incorporation of cowpea flour in wheat flour significantly reduced the score for the volume of bread. **Mcwalter *et al.* (2004)** and **Hesahm *et al.* (2007)** also reported that incorporation of cowpea flour in dough had a certain negative effect on bread volume.

Color of crust

The scores assigned to crust color of bread were significantly affected by the supplementation of mushroom powder. It is obvious from results that color scores for bread differ significantly due to the different treatments. The interaction of treatments showed a significant effect on the color score of the bread. A significant decrease in color score was observed with increase in the level of supplementation of mushroom powder. The effect of blending, on the bread color was more pronounced when higher concentrations of mushroom powder were used. The mean score for the color of the bread prepared from the mushroom powder supplemented composite flour samples are shown in Table 8. It is obvious from results that bread prepared from the flour containing 15% mushroom powder got the lowest color score (4.0) followed by the 12%

mushroom powder (4.28). The highest score (8.14) was obtained by control followed by 2% mushroom powder. It was observed that decrease in color score of mushroom powder supplemented bread decreased proportionally with the increase in the level of mushroom powder. Crust color of the bread was light brown which darkened progressively with the increasing level of mushroom powder. The darkened color of crust may be due to the Maillard reaction taking place during baking of loaves, due to high lysine contents. This corroborates with **Hussain (2004)** who found that there was a progressive decrease in assigning the scores to crust color of bread as the wheat flour was replaced by non-wheat flour. **Okafor *et al.* (2012)** also reported that scores for color of crust decreased by increasing the level of mushroom powder form control (100% wheat flour) to T_5 (15 % mushroom powder)

Symmetry of form

The results for the symmetry of form of bread prepared from different composite flours indicated significant differences among bread prepared from mushroom powder supplemented bread with respect to their symmetry of form. The mean scores assigned to the symmetry of form of bread prepared from mushroom powder supplemented bread are shown in Table 8. It is evident from the results that bread prepared from 100% wheat flour got significantly the highest scores (3) but non-significantly different from each other for their symmetry of form from the judges. The lowest (1.0) scores for symmetry of form were obtained from bread prepared with 15% mushroom powder supplemented wheat flour. **IQBAL** (2007) also found that incorporation of cowpea flour in wheat flour significantly reduced the score for symmetry of form of bread.

Evenness of bake

It is obvious from the results that evenness of bake differs significantly due to different treatments. The mean squares concerning scores allocated to evenness of bake of bread prepared from different mushroom powder supplemented wheat flours are presented in Table 8. The results point out that score for the evenness of bake of bread ranged from 2.71 to 1.42. The highest score (2.71) for the evenness of bake was allocated to the bread prepared from the 100% wheat flour (control). The bread prepared from the 15% mushroom powder supplemented wheat flour obtained the lowest score (1.42) for the evenness of the bake. **Chavan *et al.* (1991)** observed that the score assigned to evenness of bake decreased as the supplementation level of peanut flour increased. **Hussain (2004)** found that there was a progressive decrease in assigning the scores to evenness of bread as the wheat flour was replaced by non-wheat flour.

Character of crust

The scores assigned to the character of crust were significantly affected by the addition of mushroom powder in wheat flour. The mean scores assigned to the crust character of bread prepared from mushroom powder supplemented wheat flour are given in Table 8. The character of crust scores was allocated maximum (2.85) to bread prepared from 3% mushroom powder supplemented in wheat flour followed by bread prepared from 6% mushroom powder supplemented wheat flour. **Chavan *et al.* (1991)** observed that the score assigned to crust character of bread decreased as the supplementation level of peanut flour increased. **Hussain (2004)** equally reported that the scores assigned to the character of crust were significantly reduced by the addition of flaxseed flour in straight grade flour.

Color of crumb

The results indicate that mushroom powder supplementation level significantly affected the crumb color of the bread. The scores assigned by the panelists to the crumb color of bread prepared with different levels of mushroom powder supplementation in wheat flour (Table 8) indicated that bread prepared without mushroom powder supplementation (100% wheat flour) got significantly the highest scores (8.14) for crumb color while the bread prepared with 15% mushroom powder supplemented wheat flour were ranked at the bottom (3.50) by the judges. It is also evident from the results that bread prepared from 3% and 6% mushroom powder supplemented wheat flour had close values. These results are similar to the finding of **Okafor *et al.* (2012)** who observed that scores assigned to the crumb color of bread decreased as the level of mushroom powder increased. **Chavan *et al.* (1991)** observed that the score assigned to the color of the crumb of bread decreased as the supplementation level of peanut flour increased. The darkening of color is due to the Maillard reaction.

Table 8 Effect of mushroom powder on external characteristics of mushroom powder supplemented bread

Treatment	Volume	Color of crust	Symmetry of form	Evenness of bake	Character of crust	Color of crumb
T_0	7.85^{a}	8.14^{a}	3.00^{a}	2.14^{b}	2.71^{b}	8.14a
T_1	6.71^{b}	7.85^{b}	2.42^{b}	2.14^{b}	2.85^{a}	7.71b
T_2	6.00^{c}	7.00^{c}	2.14^{c}	2.71^{a}	2.71^{b}	7.00c
T_3	5.42^{d}	5.71^{d}	2.00^{d}	2.00^{c}	2.00^{c}	6.00d
T_4	4.42^{e}	4.28^{e}	1.14^{e}	1.71^{d}	1.71^{d}	4.50e
T_5	3.20^{f}	4.00^{f}	1.00^{f}	1.42^{e}	1.42^{e}	3.50f

Internal characteristics

Grain

The statistical analysis indicated that scores given to grain of bread were affected significantly by the levels of mushroom powder supplementation in wheat flour. The results presented in Table 9 indicated that there was a progressive decrease in scores assigned to the grain of bread as the supplementation level of mushroom powder increased. The highest scores (9.71) were given to grain of bread prepared from control (100% wheat flour) followed by bread prepared from 3% mushroom powder (8.71) and 6% mushroom powder (7.50) supplemented wheat flour. The lowest scores to grain (4.00) were assigned to the bread prepared from 15% mushroom powder wheat flour. These observations are in line with those made by Hussian (2004) who observed that scores given to grain of bread were affected significantly by the levels of flaxseed supplementation in straight grade flour. **Hesham *et al.* (2007)** also found that mushroom powder and legume flour deteriorates the crumb grain in proportion to quantity of flour used to replace the wheat flour.

Table 9 Effect of mushroom powder on internal characteristics of mushroom powder supplemented bread

Treatment	Grain	Aroma	Texture	Taste
T_0	7.42^{c}	7.50^{a}	8.85^{d}	13.14^{a}
T_1	8.71^{b}	7.42^{a}	11.00^{b}	11.71^{b}
T_2	9.71^{a}	7.28^{ab}	10.71^{b}	10.58^{c}
T_3	7.50^{c}	7.14^{b}	11.71^{a}	10.57^{c}
T_4	5.50^{d}	5.71^{c}	9.28^{c}	8.00^{d}
T_5	4.00^{e}	5.00^{d}	7.14^{e}	7.28^{e}

Legend: T_0 = Control (100% Wheat flour), T_1 = 3% mushroom powder supplementation, T_2 = 6% mushroom powder supplementation, T_3 = 9% mushroom powder supplementation, T_4 = 12% mushroom powder supplementation, T_5 = 15% mushroom powder supplementation

Aroma

The results pertaining to analysis of variance relating to the aroma of bread prepared from different levels of mushroom powder supplementation showed that supplementation levels significantly affected the scores given to aroma of bread. The scores assigned to the aroma of different breads presented in Table 9 showed that bread prepared from 100% wheat flour got statistically the highest scores (7.50) for aroma followed by bread from 3% mushroom powder supplemented wheat flour (7.42) whereas the minimum aroma scores were assigned to the bread prepared from 15% mushroom powder supplemented wheat flour. **Hussain (2004)** observed that scores given to aroma of bread were affected significantly by the levels of flaxseed supplementation in straight grade flour.

Texture

Sensory demonstration of food products structure in terms of their response to stress by senses in the muscles of hands, fingers or tongue in the tactical nerves in the surface of the skin is called texture **(Dah, 2010).** It is considered a quality attribute associated with product freshness. Food texture may be extremely important because it acts as an indicator of food quality. The analysis of variance regarding the texture of bread prepared from different composite flours is given in Table 9. The results indicated that scores given to texture of bread differed significantly due to differences in supplementation level of mushroom powder in wheat flour. Results proved that the bread from 100% wheat flour got the maximum scores for texture (11.71) and the scores decreased gradually as the supplementation level of mushroom powder increased in wheat flour. The bread prepared from 15% supplementation of mushroom powder in wheat flour obtained the lowest scores (7.14) by the judges for texture. **Hussain (2004)** found that there was a progressive decrease in assigning the scores to evenness of bread as the wheat flour was replaced by non-wheat flour. **Ory and Conkerton (1983)** also observed that the incorporation of peanut flour in wheat flour significantly affect the texture of bread. **Chavan *et al.* (1991)** observed that the score assigning to evenness of bake decreased as the supplementation level of peanut flour increased.

Taste

The major component of the flavor detected by the taste buds of the tongue, mouth membrane and influenced by the texture, flavor, and composite of food products is called taste. It is considered the most important attribute regarding the quality of any food product. **(Miyaki *et al.*, 2015).** The statistical results for scores allocated to the taste of the bread samples prepared from different mushroom powder wheat flour blends showed a significant effect of level of mushroom powder supplementation. The results showed that the bread prepared from 100% wheat flour got the highest scores (13.14) for taste by the panelists. It is clear from the results that bread prepared from 10% mushroom powder supplemented wheat flour were graded at the bottom with respect to taste scores. The results in Table 9 further exposed that bread prepared from 6% (10.58) and 9% mushroom powder (10.57) supplemented wheat flour acquired non-significantly different scores for taste. There was a decline in assigning the scores to bread by increasing the level of mushroom powder supplementation in wheat flour. **Okafor *et al.* (2012)** reported that scores allocated to the taste of bread decreased as the level of mushroom powder supplementation increased. **Hussain (2004)** also observed that scores given to taste of bread were affected significantly by the levels of flaxseed supplementation in straight grade flour.

CONCLUSION

The results of the present study suggest that mushroom powder may be supplemented up to 6% in wheat flour to get acceptable bread with improved protein content. The blending of mushroom powder in wheat flour can help to improve the nutritional status of the masses in the under-developed and developing countries.

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