



## Production of bio-flavored sourdough bread

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### Abstract

Sourdough is the oldest form of leavened bread and has been used since the time of the pharaohs. Sourdough is a mixture of flour and water that is fermented by naturally present Lactic Acid Bacteria (LAB) and yeast. With modernization and an increase in demand, the bread industry currently produces easy to leaven soft bread using Baker's yeast. To get a sourdough bread with consistent flavors and texture is considered to be an art and skill. The purpose of our endeavor has been to bring in scientific principles and the use of a laboratory-developed defined consortium for better and reproducible sourdough product with consistent flavor, texture and aroma. Of the twelve isolates obtained from selected probiotics, four organisms: *S. boulardii*, *L. acidophilus*, *L. rhamnosus* and yeast strain isolated from Kefir were selected based on their production of flavoring compounds. The individual ability of each organism to impart better flavor and texture to the bread product was assessed using standard flavor and texture profiling. Five tasters were provided with a rubric to evaluate the flavor as well as the texture of the bread. In-house kilning apparatus—for the malting of flour and texture analyzer was designed for texture profiling.

**Keywords:** Sourdough Bread, Solid State Fermentation, Lactic Acid Bacteria (LAB), Yeast, Bio-flavor, Probiotic

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## 1. Introduction

Sourdough is a form of leavened bread which many believe was invented by the Egyptians, to more than 5,000 years ago. Initially, sourdough batters were a simple mixture of flour and water that were fermented and were then used as leavening to make bread rise. It was observed that this process could be expedited by using a starter culture (also known as a leaven)—a mixture of flour, water and naturally occurring bacteria and yeast which may be kept indefinitely, if stored and fed properly. Only a small portion of the starter is used to make bread by mixing with a large portion of flour and a little bit of water (Back-slop method).

Before the mechanism was understood, the unknown gas producers were called “seeds” (Couch, 2016). By 1910, traditional sourdough was much less common because bread made with commercial yeast was much faster and easier, and produced a more consistent product. The positive qualities of sourdough bread were unfortunately overlooked because of the convenience that commercial yeast offered (Katina, 2005). Recently,

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it has been rediscovered thanks to several effects on organoleptic, nutritional, and functional features of cereal-based products. Acidification, proteolysis, and activation of endogenous enzymes cause several changes during sourdough fermentation, carried out by lactic acid bacteria and yeasts, which positively affect the overall quality of the baked goods. The whole wheat flour processed with yeast proteases and selected lactic acid bacteria was demonstrated to be safe for coeliac patients (De Vuyst and Neysens, 2005; and Koceva Komlenić et al., 2012).

The texture of a food is contributed by its composition, chemical or biochemical processes that the food undergoes. In case of Sourdough, most of the contribution to the texture depends on the organism present, fermenting the available substrates inside the dough to produce acids or gases. As a result, controlling and ensuring the organism undergoing solid-state fermentation of the dough would, to a large extent, result in controlling the resultant texture of the bread. Sourdough flavor components identified as produced by metabolic interactions between yeast and LAB fall into many classes of compounds. It is generally considered that in sourdoughs, the ratio of LAB to yeast should be 100:1 for optimal activities. LAB have various role in Sourdough fermentation such as carbohydrate metabolism, protein metabolism, development of aroma compounds. (Nionelli et al., 2016; and Salim-ur-Rehman et al., 2006).

Apart from the microbial composition, the texture of the Sourdough bread depends on the constitutive composition of the dough i.e., concentration of certain substances like protein (e.g., Gluten), sugars, water etc. Bread sensory profile can be described by appearance, structure, texture and flavor. The same quality parameters are generally determined instrumentally and are evaluated by descriptive sensory analysis (Curi et al., 2011). Probiotics are live microorganisms that are intended to have health benefits when consumed (Lye et al., 2016 and Wilhelm et al., 2001). Use of these organisms to ferment the bread would have flavor benefits; as certain probiotic organisms are known for their ability to produce flavoring compounds such as lactic acid, acetaldehyde, diacetyl, etc. by the process of biotransformation.

## 2. Materials and methods

### 2.1. Probiotic organism – Treatments, isolation, characterization

#### 2.1.1. Samples

Probiotic foods, commercially available capsules and sachets were used for isolation of the organisms and subsequently used in a making of bread. The desired organisms were isolated from the mentioned sources.

*S.boulardii* - Dr. Reddy's Econorm™ sachet; *S.cerevisiae* - Bluebird® Baker's Yeast; *L.acidophilus* - Dr. Reddy's Becelac Fortz; *L.casei* Shirota - Yakult® Danone Probiotic drink; *Leuconostoc mesenteroides* – ATCC culture; *L.paracasei* - Sundyota Numandis Remune AI; *L.rhamnosus* GG- Mystical Biotech Pedigut GG; *L.sporogenes* - Cipla New Nutrolin B plus; *Streptococcus thermophilus* & *L.bulgaricus* - Drums Food Epigamia Greek Yoghurt; Kéfir organisms - Probiotics Food Kéfir culture (with organic cow's milk). Certain probiotic samples were subjected to conditions that would mimic pH conditions of the human gut for improving isolation, as they failed to show viability when directly plated.

#### 2.1.2. Media

Microbiological media, or bacterial culture media, is a growth medium used to grow bacteria (gibraltarlabsinc.com). The cultures were isolated from the probiotic sources mentioned, characterized and preserved before use. All chemicals used for media preparations were of GR grade.

deMan, Rogosa and Sharpe media (MRS media), 10% Milk Agar medium, Tomato juice agar (TJA) and *Streptococcus thermophilus* Isolation Media (STI media) was used for isolation of microorganisms from probiotic samples. Also, a modified tomato juice agar (mTJA) media was used which was modified by our laboratory previously to prepare a cheaper and more suitable media for detection of LAB. Yeast extract powder a media component in original TJA was replaced by vitamin B capsule (Omega B-complex capsule - Omega Pharma Pvt. Ltd.) and casein enzymatic hydrolysate is replaced by milk powder (Nestle India Ltd.) which acts as alternate source of nitrogenous compounds and amino acids. Use of milk powder and vitamin tablets not only reduces the cost of media but also allows for better results obtained upon culturing.

A medium called 'Flour medium' was prepared, 2% flour in distilled water, to demonstrate the organism's growth in bread loaves. It was used to check whether organisms show similar growth metabolites as in MRS broth.

### 2.1.3. Characterization

Each organism exhibits different characteristics with respect to shape, size, arrangement, colony morphology, Gram nature, presence or absence of capsule and endospores, ability to utilize various energy source (Karen, 2012). These characteristics help in the characterization of different organisms.

### 2.1.4. Detection and estimation of flavoring compounds and organic acids

#### 2.1.4.1. Influence in flavor: Determination by IMViC and Urease test

IMViC and Urease, along with a couple of other tests are frequently used as gold standard for identification of *Enterobacteriaceae*. In this research however, IMViC and Urease test were used to determine the influence of flavor by the probiotic organisms used. Indole test was used to determine production of indole upon utilization of tryptophan which contributes to undesirable floral notes in low concentrations and putrid smell in sourdough when present in high concentrations (Purves *et al.*, 2001). Methyl Red test was used to determine high acid and low acid producers which directly contributes to the sourness of the sourdough bread (Sylvia, 2019). Vogues-Proskauer test was used to determine ability to produce acetoin, responsible for buttery taste, via carbon metabolism (Sylvia, 2019). Citrate Utilization test was used to determine ability to utilize citrate as a carbon source, allowing glucose-citrate co-fermentation, increasing acetoin-diacetyl yield, if produced (Jyoti *et al.*, 2003). Urease test helped to determine ability to break down urea, which is extensively used as foliage spray pesticides, into carbon dioxide and ammonia, latter of which is not desirable in sourdough bread (Clay and Carlson, 2010).

#### 2.1.4.2. Estimation of total acids by Titrimetric method

Titrimetry against standard NaOH was used to estimate total amount of acids produced by organisms in different media.

Method- Four different media - MRS (Protein media), Glucose Peptone Media, 2% Refined wheat flour and 2% Whole wheat flour, were used for determining the most suitable media for acid production. 24hr incubated culture broth were centrifuged and supernatant was titrated against NaOH standard.

#### 2.1.4.3. Chromatography techniques

- A) Thin layer Chromatography: Classical silica TLC plates (Analytical Chromatography TLC Silica gel 60 F<sub>254</sub>, 25 Aluminum sheets 20 × 20 cm, Merck KGaA, Darmstadt Germany) coupled with UV-Visualization was used to detect the presence of organic acids and benzaldehyde in the samples using Ammonia: Absolute Ethanol (7:13) solvent system. 10% organic solution standards were used to determine and compare Rf respectively (Upadhy *et al.*, 2009 and Wilson *et al.*, 2010). Methodology followed in our studies was adapted from Lisa (2019).
- B) Gas Chromatography: Agilent Db-1 GC column along with FID detector was used to detect the presence of acetoin and/or diacetyl in the samples. Methodology followed in our studies was adapted and modified for acetoin and diacetyl from Gokce *et al.* (2014).

### 2.1.5. Flour Processing and Analysis

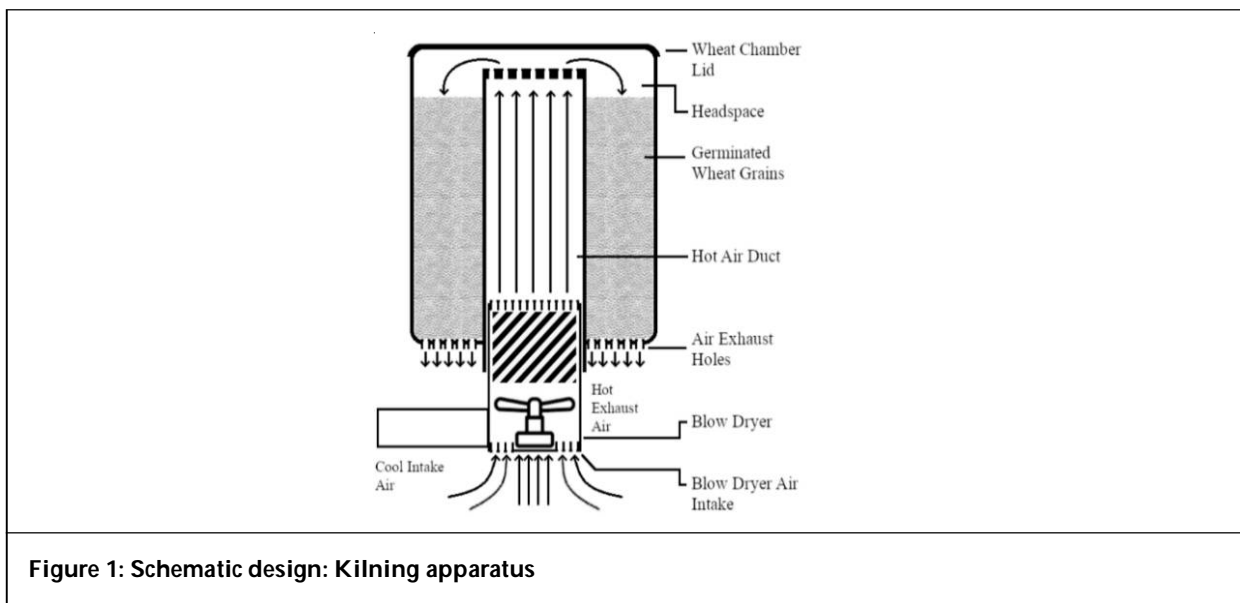
#### 2.1.5.1. Malting Process

(Method used is a modification of the procedure defined in Malts and Malting, (Briggs, 1998)

Batches of germinated wheat grains (shoot length less than 3 mm) were subjected to kilning in an in-house fabricated kilning apparatus (Figure 1). The fabricated set up used a hair/blow drier at 60°C thermostatic temperature with alternating heating and cooling cycles for a total time of 30 minutes. The grain was dried and grounded in a flour mill to get malted grain flour.

#### 2.1.5.2. Flour Analysis

Estimation of sugar content in different types of flour by DNSA method.



**Figure 1: Schematic design: Kilning apparatus**

Malting releases/increases the quantity of simple sugars. This was estimated using DNSA method.

#### 2.1.6. Starter culture and bread preparation

##### 2.1.6.1. Starter culture preparations

###### A) Development of standard starter culture:

Methodology - 17.7 g of flour was taken a clean bowl in which, 5 ml at a time (total 18 ml) water was added and kneaded. The container was covered with cling film/ lid and left undisturbed for 24 h at room temperature. After 24 h initial incubation, equal amount of flour and water was added in the culture and was mixed. This step is based on principle of back-slopping and is known as 'feeding of starter culture'. Similarly feeding was done after every 12 h (This recipe is adapted from [Obscura, 2017](#)).

###### B) Development of consortium:

In order to avoid the antagonistic interactions between the organisms, four separate starter cultures of standard volume were prepared using individual organisms. This would ensure the proper development/ growth and exhibition of the flavors by individual organisms. After 20 h of incubation, 1/4<sup>th</sup> amount of the total quantity from each individual starter cultures were mixed to develop the consortium and restore the standard volume of starter culture used for bread preparation. This mixture was then used as the starter culture for preparing the consortium bread. The consortium was mixed with the flour for bread preparation and it was kept for second leavening for 5 h before baking.

##### 2.1.6.2. Bread recipe standardization

Methodology - 53 g of flour was mixed with the starter culture; 25 ml of water was added (5 ml at a time) intermittently while kneading the dough. The dough was transferred to the greased bowl and kept for 5 hrs leavening while being covered with a wet cloth to ensure fermentation. The dough was baked at 180°C for 25 min in preheated oven.

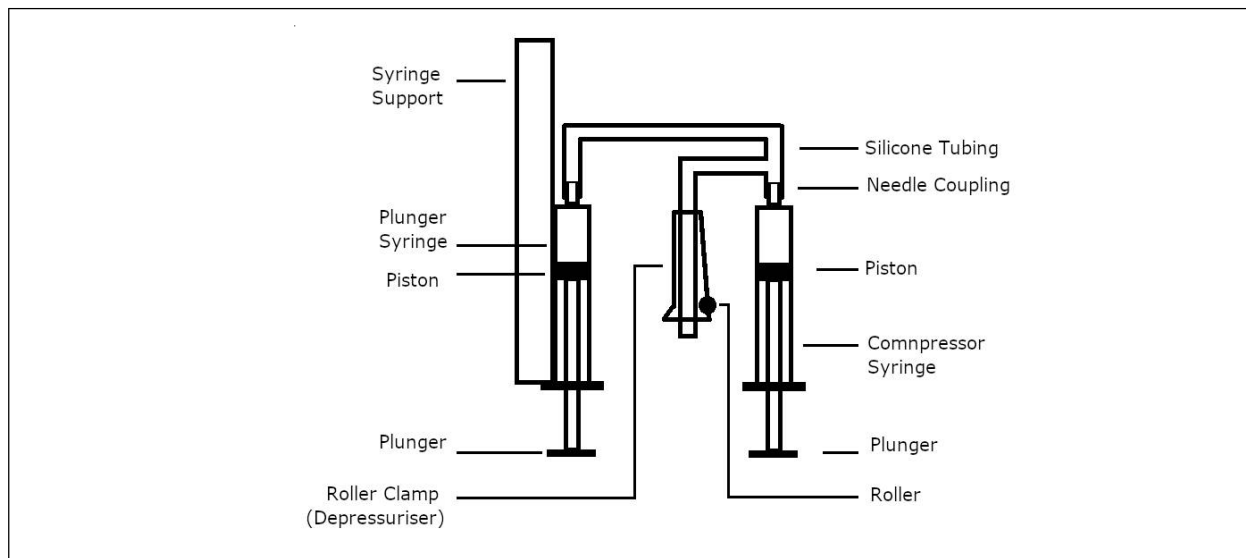
(This recipe is a modified version of <https://www.thekitchn.com/how-to-make-sourdough-bread-224367>)

#### 2.1.7. Bread profiling

##### 2.1.7.1. Texture

Textural attributes are key characteristics that consumers appreciate in bread which include the softness and sponginess of a bread. Moreover, these parameters are associated with texture are used routinely to assessed bread quality.

Commercially, bread texture analysis is done using equipments like TA-XTplus Texture Analyser (Stable Micro System, Godalming, Surrey, UK), Minolta Colorimeter (Minolta Co., Osaka, Japan) and others. ([Angioloni and Collar, 2009](#)) In this project, texture analysis limited to softness and sponginess was performed and recorded using an in-house fabricated functional replica (Figure 2).



**Figure 2: Schematic design - Texture analyser**

<b>Table 1: Rubric used for taste profiling</b>		
Name of the assessor:	Product:	
Analyst:	Date:	
Have you ever eaten freshly baked bread?		
Have you ever eaten sour dough bread?		
Parameter	Scored on a scale from 1 to 5	Name of microorganism
Flavors	5	
Bland		
Malty		
Nutty		
Alcoholic		
Buttery		
Milky		
Wheaty		
Salty		
Sweet		
Sour		
Bitter		
Yeasty		
Aroma	Descriptive	
Overall texture	10	
Overall taste	10	

### 2.1.7.2. Taste

A breads sensory profile can be described by its texture and flavor. The most important evaluation criteria of a bread were selected by a rubric (Table 2) given by 6 trained panellists according to ISO 11035:1994 and Carr and Tadini (2003) (Curic and Novotni, 2011). A simpler version of rubric was prepared for “descriptive sensory analysis” where appearance, structure and texture criteria were covered under “Overall texture score”.

The assessors were asked to determine the most pronounced flavor with their first bite, later the assessors were asked the routine questionnaire. The assessors were subjected to ‘Blind Testing’, that is, were giving a bread they have already assessed to check reliability of their assessment.

Breads were prepared as follows:

A set of 13 bread in which control was standard sour dough bread and 12 breads was fermented using dough’s having 12 different isolated microorganisms as starter culture was prepared. Data obtained from these sets were used for consortium development. Furthermore, bread was prepared using consortium along with breads having individual microorganisms which are used in consortium.

## 3. Results and discussion

### 3.1. Probiotic organism- Treatments, isolation, characterization

The micro-organisms were successfully isolated from probiotic samples using varied media and were identified using colony characteristics and biochemical tests based on Bergey’s manual and Yeast database accordingly (Burgey et al., 1975 and pathway.yeastgenome.org). Treatment of certain samples by mimicking the gut-like conditions yielded observably significant results.

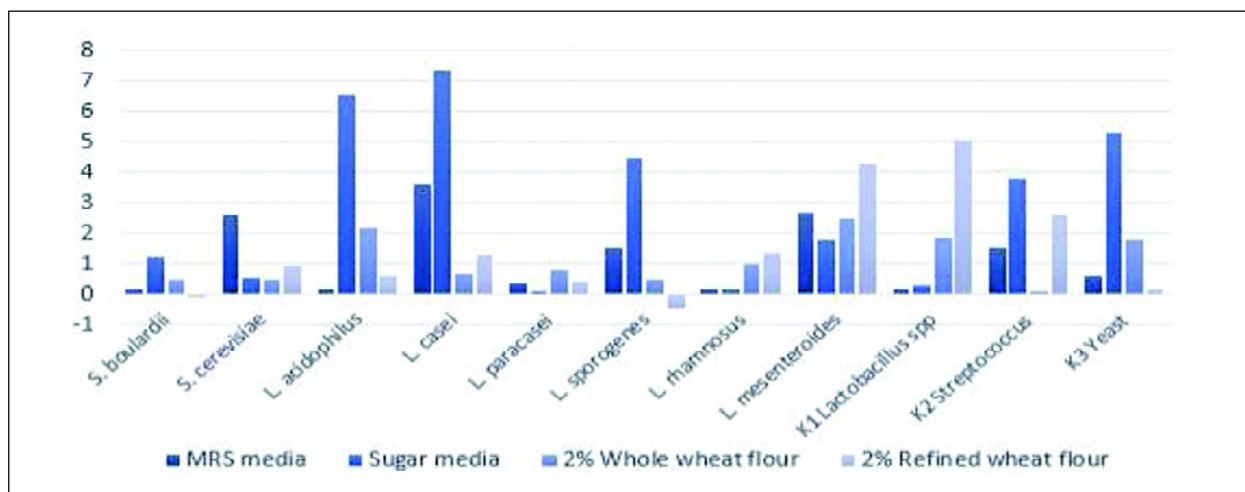
### 3.2. Detection and estimation of organic acids and flavoring compounds

### 3.3. Quantification of acid produced

The results indicate that organisms that produce acid, do so best in rich media, i.e., MRS media.

**Table 2: Influence in flavour determined using IMViC and Urease Test**

Biochemical	Urease test	Indole	Methyl Red	Voges Proskauer	Citrate	Organism
Colonies/source						
Econorm	-	-	+	-	-	<i>Saccharomyces boulardii</i>
Active Dry Yeast	-	-	-	-	-	<i>Saccharomyces cerevisiae</i>
Becelac Fortz	-	-	-	-	-	<i>Lactobacillus acidophilus</i>
Yakult- Danone	-	-	-	-	-	<i>Lactobacillus casei</i>
Epigamia	-	-	+	-	-	-
Remune AI	-	-	+	-	++	<i>Lactobacillus paracasei</i>
New Nutrolin B Plus	-	-	+	-	-	<i>Lactobacillus sporogenes</i>
Pedigut GG	-	-	-	+	-	<i>Lactobacillus rhamnosus</i>
<i>Leuconostoc mesenteroides</i>	+	-	+	-	+	<i>Leuconostoc mesenteroides</i>
K-1	-	-	+	-	+	<i>Lactobacillus spp.</i>
K-2	-	-	+	-	+	<i>Streptococcus spp.</i>
K-3	-	-	+	-	+	Yeast



Graph 1: Relative acid production by organisms in different media

2% whole wheat flour media facilitated fairly good amount of acid production with *Leuconostoc mesenteroides* being the highest acid producer followed by *L. acidophilus*. Refined wheat flour is not suitable for use as raw material for production of sourdough bread as the organisms were incapable of producing high amount of acids in it. Thus, Whole wheat flour is a suitable raw material for production of sourdough bread.

### 3.4. Chromatography Techniques

#### 3.4.1. Thin Layer Chromatography

Other than the organic acids, production of benzaldehyde instils an almond like flavor and was found to be produced by *S.cerevisiae*, *S.boulardii*, *L.acidophilus*, K2 *Streptococci* and K3 yeast. None of the inoculated broth

Table 3: TLC – Compounds produced by organisms

Standards	AC	B	C	D	L-I	L-II	MA	M	P	UK	
Standard Rf	0.96	0.89	0.17	0.96	0.59	0.81	0.24	0.48	0.55	0.36	
Organisms	<i>S. boulardii</i>	-	+(0.92)	-	-	+(0.60)	+(0.79)	-	-	-	+(0.33)
	<i>S. cerevisiae</i>	-	+(0.89)	+(0.16)	-	-	+(0.76)	-	-	+(0.56)	+(0.38)
	<i>L. acidophilus</i>	-	+(0.89)	+(0.10)	-	+(0.57)	+(0.78)	-	+(0.47)	-	+(0.37)
	<i>L. casei</i>	-	-	+(0.16)	-	-	+(0.77)	-	-	+(0.55)	+(0.36)
	<i>L. paracasei</i>	-	-	+(0.18)	-	-	+(0.80)	-	-	-	+(0.33)
	<i>L. rhamnosus</i>	-	-	-	-	-	+(0.81)	-	-	-	-
	<i>L. sporogenes</i>	-	-	-	-	-	+(0.80)	-	-	-	-
	<i>Leuconostoc mesenteroides</i>	-	+(0.91)	+(0.16)	-	+(0.57)	+(0.76)	+(0.23)	-	-	+(0.39)
	K1 Lactobacilli	-	-	-	-	-	+(0.79)	-	-	-	+(0.32)
	K2 Streptococci	-	+(0.91)	-	-	+(0.56)	+(0.76)	-	-	-	+(0.34)
	K3 Yeast	-	+(0.88)	-	-	+(0.58)	-	-	-	-	+(0.35)

Note: Legend: present = +; absent = - ; A = Acetaldehyde ; AA = Acetic Acid ; AC = Acetoin ; B = Benzaldehyde ; C = Citric Acid ; D = Diacetyl ; E = Ethyl Acetate ; L = Lactic Acid ; MA=Malic Acid ; M = Mannitol ; P = Pyruvic Acid

exhibited a spot corresponding to the Rf of these acetoin or diacetyl on the TLC plates. Hence, a more sensitive method: Gas Chromatography was employed for detection of the respective compounds if present.

### 3.4.2. Gas Chromatography

Though some of these cultures are known to produce acetoin and diacetyl, these compounds were not detected in any of selected organisms in both whole wheat flour as well as refined wheat flour media indicating absence of these compounds or presence below GC detector threshold concentrations.

Flour Processing and Analysis:

### 3.5. Flour Processing – Malting

Flour obtained from malted wheat appeared to have a darker shade, pleasant aroma and sweeter taste as compared to whole wheat flour.

#### 3.5.1. Flour Analysis – DNSA

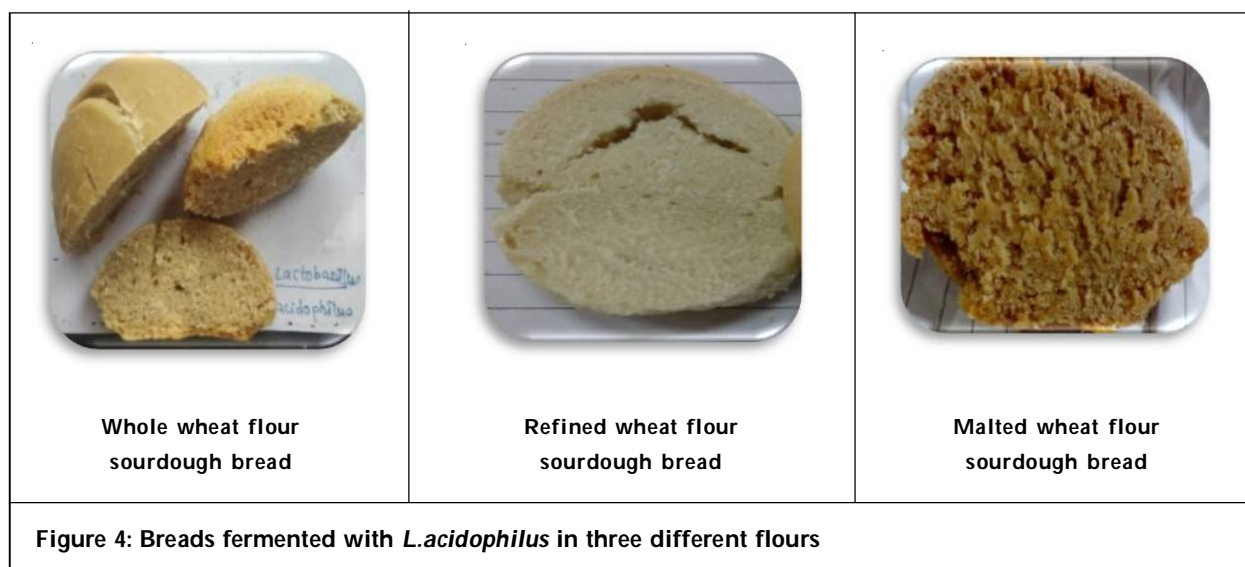
Although the malted flour gave a pleasant aroma and flavor, the texture of the bread was sticky and not an acceptable bread like chewy texture. The texture properties and mouthfeel were not appreciated by the tasting panel. Thus, further studies with the malted flour were stopped.

### 3.6. Sour dough Bread Preparation

The bread was baked and cooled before the quality of the bread was determined.

Wheat malt flour	Whole wheat flour	Refined wheat flour
= 3.8 g%	= 1 g%	= 0.2 g%

#### 3.6.1. Bread Profiling



#### 3.6.2. Texture

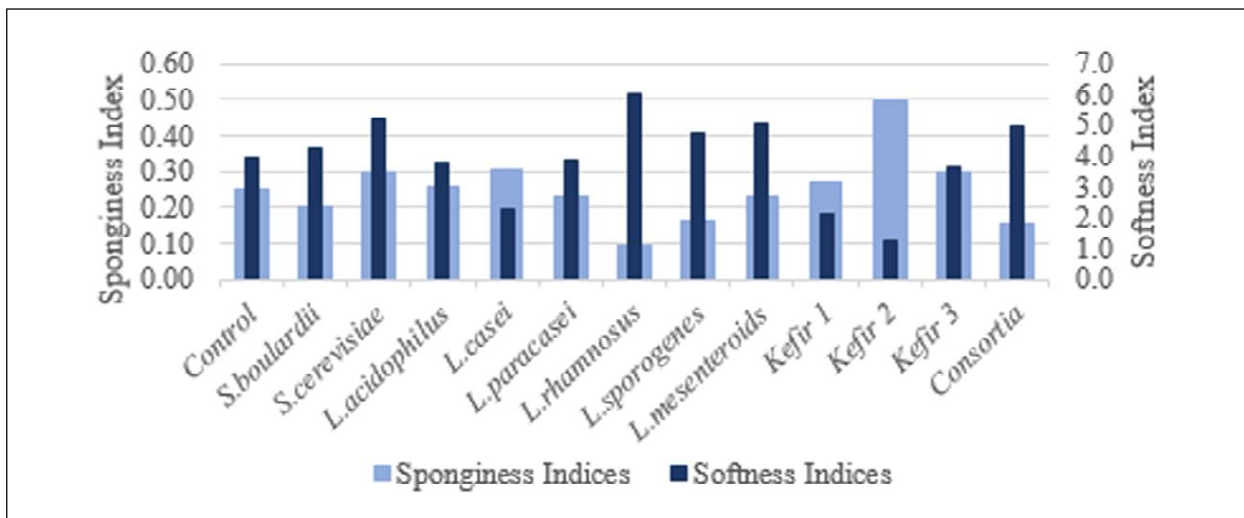
The in-house fabricated texture analyzer was used to determine softness and sponginess indices of the breads prepared which were compared amongst each other. This allowed determination of individual contribution to the bread texture of the consortium organism (Graph 2).

#### 3.6.3. Taste

The tasting was recorded using a flavor rubric (Table 2). The analysts asked specific questions to help in differentiate and emphasize various probable flavors of a bread.



Breads prepared using *S.bouardii*, *L. rhamnosus* and K3 Yeast obtained the highest scores with respect to the texture as this yeast produces comparatively higher CO<sub>2</sub>, than even *S.cerevisiae* which is quite often preferred for industrial level bread production. Resulting in better leavening of the sourdough, hence contributing to the softness and sponginess of the sourdough bread in the mixed consortia.



Graph 2: Texture attributes by different organisms

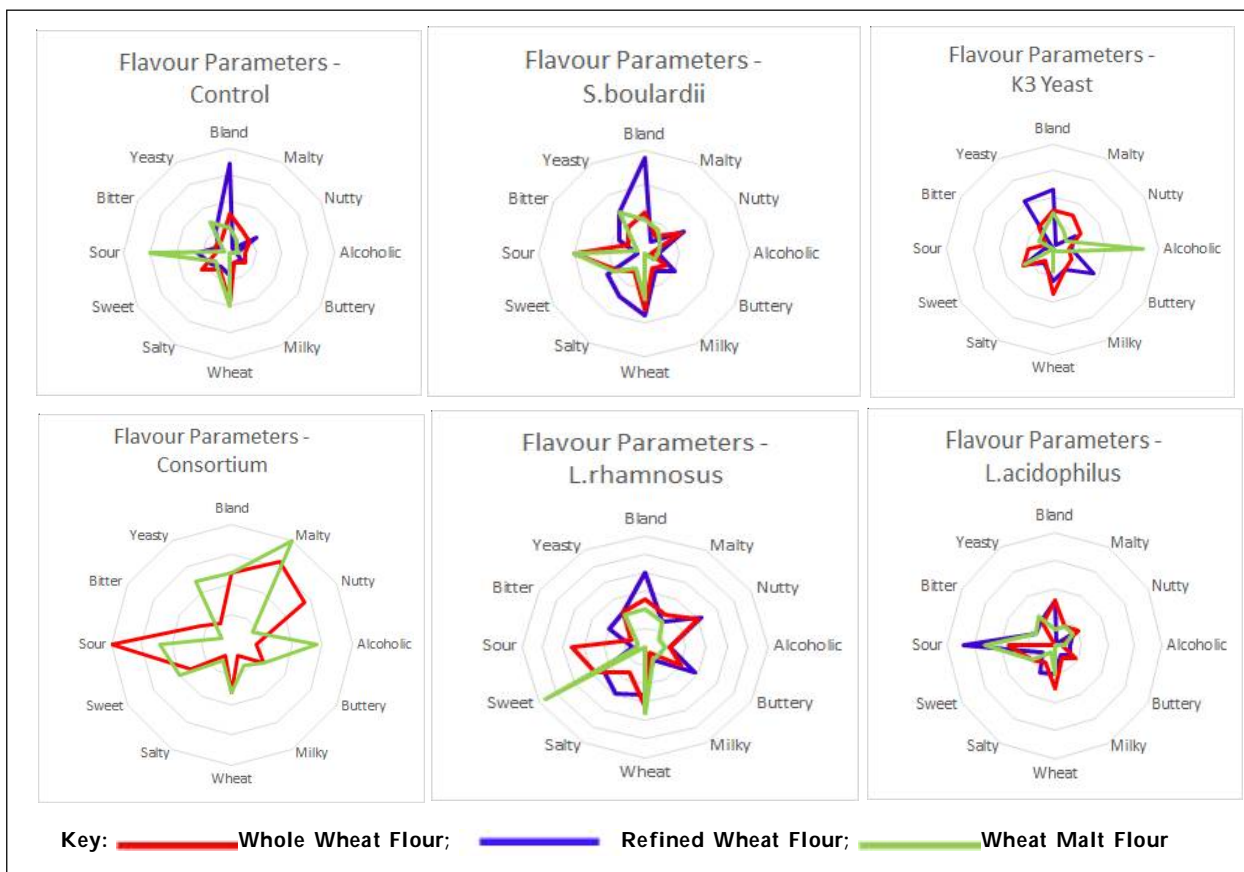
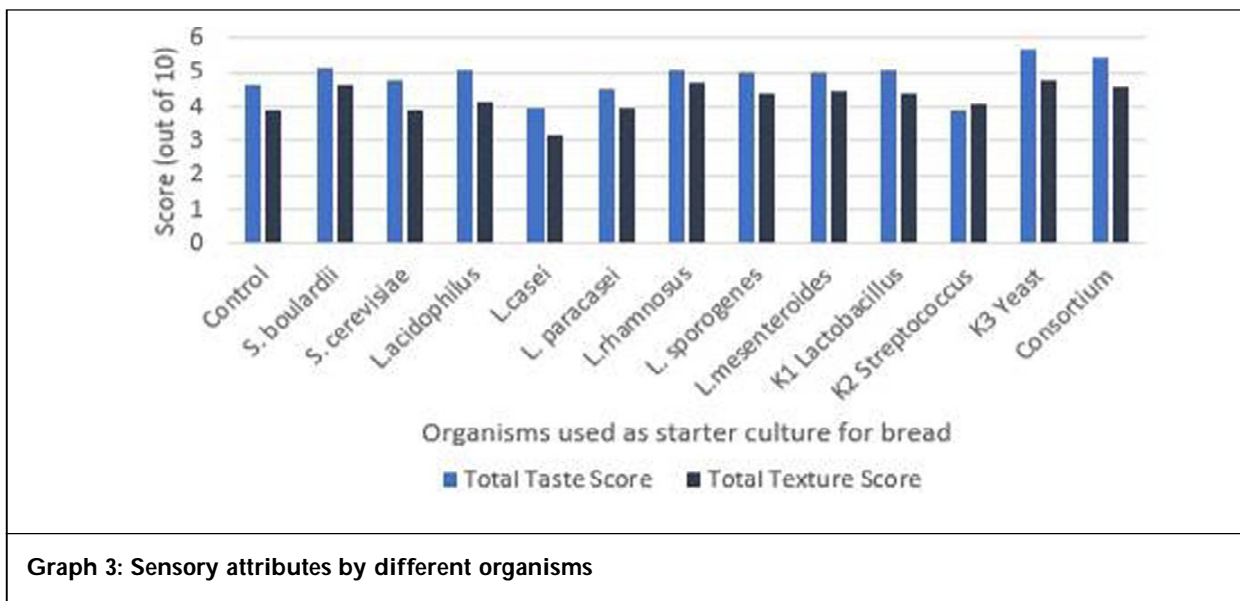


Figure 3: Flavor Parameters

Average of total taste scores (Graph 3) was found to be highest for bread prepared using K3 Yeast as starter culture followed by breads having *S. bouardii*, *L. rhamnosus* and *L. acidophilus* as starter culture. Thus, 4 microorganisms, that is, *Saccharomyces bouardii*, K3 Yeast, *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* were selected based on the data obtained from bread profiling of all the breads prepared using individual microorganisms.



#### 4. Conclusion

Out of the twelve organisms selected that included yeasts and lactic acid bacteria, ten organisms were successfully isolated from probiotics food and supplements and were identified. The organic acids produced by microorganisms were estimated using total acid titration, *Leuconostoc mesenteroides* and *L.acidophilus* were the highest acid producers in whole wheat flour media. Various other flavoring compounds produced by individual organisms were identified using chromatographic techniques. The results of TLC revealed that all organisms under study were producing lactic acid, while other compounds like citric acid, benzaldehyde, pyruvic acid were produced by only some organisms. Some organisms even produced some unknown compound which could not be identified. It was inferred from the results of GC that, none of the organisms produced acetoin or diacetyl. The DNSA test was performed to measure the amount of reducing sugar present in the different types of flour being used for bread preparation. The sourdough breads fermented with these individual organisms, that received highest scores and best compliments from the tasting panel were selected for final consortium, i.e., *S.bouardii*, *L.acidophilus*, *L.rhamnosus*, K3 yeast isolate. The sourdough bread fermented using this consortium when compared with the individually fermented bread for the overall appeal. Texture analysis of whole wheat breads revealed differences in softness and sponginess index of the consortium sourdough bread when compared with commercial yeast breads (White Bread) - lead to variation in the mouthfeel of the two breads. In the taste profiling of whole wheat sourdough breads, three out of the five members of the tasting panel affirmed that they would consider buying a sourdough bread with the characteristics of the consortium bread, if such a product were ever available in the market. The tasting panel also graded the consortium breads higher than both the control bread samples for both taste and texture. Based on responses from tasting panel, wheat malt flour was not found to be a suitable component for making consortium sourdough bread as the bread texture was not appealing.

Hence, a sourdough bread made with whole wheat bread and fermented using the formulated consortium exhibited scrumptious taste and a contrasting texture. This could provide the consumers with a healthier, tastier and feasible alternative for the commercial yeast and sourdough breads currently available in the market.

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