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Kluyveromyces marxianus Prepared as a Ready to Use Supplemental Food (RUSF)

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Abstract

Ready to Use Supplemental Food (RUSF) is a nutrient dense paste or compressed bar used to supplement a person's nutritional needs because of malnutrition or due to food shortages. The purpose of this article is to demonstrate some methods of how the dairy organism Kluyveromyces marxianus can be used to enrich the protein value of bread or ferment a substrate such as wheat bran into a more digestible form.

Introduction

The purpose of this article is to demonstrate how the dairy organism Kluyveromyces marxianus may be used to supplement the nutritional properties of human food. Through the fermentation process, enzymes are released breaking down substrates such as wheat bran into a more digestible form. The second pathway is by accumulating amino acids from the substrate and growing biomass. It is with this pathway whey can be processed into a high protein material without the need for a membrane system. One justification is K. Marxianus enriches the amino acid value of a food material, therefore it may be used as an alternative to soybean meal.

Background

The recommended management strategy of Moderate Acute Malnutrition (MAM) according to the World Health Organization is: [1]

1. Optimal use of locally available nutrient dense food.
2. The use of a variety of approaches to ensure the adequate intake of nutrients.

Unfortunately, some people do not have the money to purchase animal products such as meat and milk on a regular basis. Also, supply chain

problems, shortages and emergency situations may limit the availability of nutrient rich foods. Ready to use Therapeutic Foods (RUTFs) and Ready to use Supplementary Foods (RUSFs) are energy and nutrient dense products that are formulated as lipid pastes or compressed bars that contain high quality protein, fatty acids and micronutrients. Most formulations contain:

- Legumes such as peanuts, lentils or soybeans
- Cereal grain such as corn, sorghum, or rice
- Sugar
- Dried skim milk, whey permeates, or whey protein concentrate
- Chickpeas
- Sesame seeds
- Almonds

All formulations contain vitamins and minerals to fortify the nutritional value of the final product. RUTFs has enabled the treatment of Severe Acute Malnutrition (SAM) and MAM. Frequently RUTF and RUSF products have been designed to have a long shelf life and resist bacterial growth. RUSF are used to provide 500 kilocalories of a person's diet a day. [1]

Challenges to implantation: [1]

1. The availability and cost of the ingredients. Some ingredients are season specific or depending on the agricultural output of the time.
2. The cost of the RUTF or RUSF may not be competitive with other foods or imports. Some ingredients such as dairy products and vitamin premix are expensive and limit their use.
3. The production process must be able to maintain adequate sanitation and hygiene required for a high value product. Also, the facility needs to avoid bacterial and fungal growth.
4. The bioavailability and level of antinutrients within the ingredients needs to be considered.
5. Local production requires a well-designed business model so a factory can be built. What scale of a factory will be needed to fulfill the nutritional requirements of the population is another question that needs to be answered.
6. Technical knowledge required to produce and package the final product will be needed.
7. The producer will need to know how the consumer will use the final product and what considerations will be needed for a high level of acceptance.

The industrial production of Kluyveromyces marxianus

Whey that has been filtered through a membrane to remove the protein value is called deproteinized whey. A lactose content of 50 g L^{-1} corresponds to a Chemical Oxygen Demand (COD) between 40,000 and 60,000 parts per million (ppm). A high COD such as this places a large strain on wastewater treatment plants. In this section the cultivation of a common dairy organism Kluyveromyces marxianus (also known as Kluyveromyces fragilis) was evaluated as a source of protein. [2]

Cultivation

The organism used was K. marxianus CBS 6556 [taxonomic synonym, Kluyveromyces fragilis (A. Jörgensen) Van der Walt] was obtained from the Centraalbureau voor Schimmelcultures (CBS, Delft, the Netherlands). [2]

Media used: [2]

1. yeast–malt (YM) medium with the following composition: 5 g / l peptone, 3 g / l malt extract, 3 g / l yeast extract, 10 g / l glucose, 20 g / l agar
2. 300 ml sweet whey contained 5 g / l agar
3. 300 ml sour whey contained 10 g / l agar

Deproteinized sweet whey concentrates (DWC 20, 20% dry weight after water evaporation) were obtained from Milei GmbH (Leutkirch-Adrazhofen, Germany). DWC 20 contained approximately 120 g / l lactose, 2.5 g / l Llactate, 400 mg / l NH4 + (NH4)2SO3, 1.2 g / l calcium (pH ~5.6)

Deproteinized sour whey concentrate (140 g / l lactose, 28 g / l L-lactate, 400 mg / l NH4 + (NH4)2 SO3, 4.8 g / l calcium, pH ~4.5) and sour whey concentrate (198 g / l lactose, 13 g / l L-lactate, 1.2 g / l NH4 + (NH4)2SO3,3g / l calcium, pH ~5.5) were obtained from Nordmilch eG (Edewecht, Germany) [2]

The whey was sent through a crossflow and sterilization filter before the beginning of the operation. [2]

Culturing process : [2]

1.The process of culturing began in petri dishes.

2. Preculture 1 was made in a 250-ml shaking flask with four baffles and filled with 100 ml whey. One inoculation loop of K. marxianus CBS 6556 was added.

3. After inoculation, the 250-ml shaking flask was incubated for 24 h with agitation at 75 rpm.

4. Preculture 2 was made in a 1,000-ml shaking flask with four baffles and filled with 400 ml whey.

5. For the inoculation of preculture 2, 5 ml of preculture 1 was used followed by incubation of the 1,000-ml shaking flask for 16 h with agitation at 75 rpm.

6. For sour whey and deproteinized sour whey a 1,000-ml shaking flask was incubated for 24 h with agitation at 75 rpm.

7. For sour whey processes 40 ml of preculture 1 was added to preculture 2.

Bioreactor process [2]

1. K. marxianus CBS 6556 was cultivated at 30°C in a 30-liters or 100-liters stirred bioreactor equipped with a pH, aeration, weight, temperature, and agitation control (Bioengineering, Wald, Switzerland).
2. The pH adjustment was made with 10% (w/w) NaOH (or 10% H3PO4 in the case of sour whey) and 25% (v/v) ammonia solution (NH3) and kept at 5.8 in the case of sweet whey or 4.8 in the case of sour whey.
3. The pO2 value was adjusted maximally to 50% and minimally to 10%. The mixing speed was 200–1000 rpm and the airflow was 11–15 N l/min.
4. The medium consisted of 13.5-l or 55-l filter-sterilized deproteinized sweet whey concentrates (DWC 20).
   Furthermore, 1.5-l or 5.5-l of K. marxianus CBS 6556 preculture 2 was used to inoculate the reactor.
5. The total volume added into the reactor was 15 or 60 l, respectively. Deproteinized sour whey concentrate was similarly used for the cultivation in a 30-l scale.

The cultivation of K. marxianus on DWC 20 without any supplementation ended growth at a dry biomass concentration of 25 g / l with 80 g / l lactose remaining in the medium.

The optimal nutrient supplementation for sweet whey was 500 mg / l $(NH_4)_2SO_4$ 1 g / l $CaCl_2$ and 2.5 ml / l trace element solution.

The optimal supplementation of sour whey was 1.5 g / l $NH_4)_2SO_4$ and 2.5 ml / l vitamin and 1 ml l trace element solution.

The maximum biomass of sweet whey is 50 g / l and 65 g / l for sour whey. [2]

Pure sweet whey has a COD of about 150,000 mg / l. After fermentation with K. Marxianus this value was reduced by a factor of ten to 15,000 mg / l. Sour whey COD was reduced from about 193,000 mg / l to 32,533 mg / l or a 83% reduction. [2]

Table 1. [2]

Amino acid	Advice of WHO	DWC 20	SCP (DWC 20)	Deproteinized sour whey	SCP (deproteinized sour whey)
Valine	5	4.5	6.89	6.82	7.5
Leucine	7	5.07	7.62	5.82	7.74
Isoleucine	4	3.19	5.07	6.71	5.48
Methionine	3	0.73	0.95	1.11	0.77
Phenylalanine	6[a]	1.95	3.67	2.42	3.58
Tyrosine	6[a]	0.85	2.45	1.52	2.5
Threonine	4	5.47	7.45	9.2	6.94
Histidine	5.5	1.8	2.08	1.87	1.9
Tryptophan	1	ND	ND	ND	ND

Amino acid composition (g/100 g protein) of sweet and sour whey in comparison to the amino acid composition of Single Cell Protein (SCP) produced from sweet and sour whey and to the guidelines of the World Health Organization (WHO). a: The recommendation is for the concentration of phenylalanine and tyrosine together. ND: not determined.

As can be seen in the table above, the amino acid values were increased by K. marxianus fermentation. Only 4 of the amino acids that were evaluated are below the World Health Organization (WHO) for human health. [2]

Kluyveromyces marxianus Versus Commercial yeast in bread production

Cultivation of Kluyveromyces marxianus [3]

1. K. marxianus (NBRC 1735) was provided by NITE Biological Resource Center (Kisarazu, Chiba, Japan)

2. Culture conditions: Grown at 30 °C in the culture medium containing 10 g/L of lactose, 5 g/L of peptone, 3 g/L of yeast extract and 3 g/L of malt extract.
3. The culture was washed twice with sterilized water and used for bread making.

Bread making process for Kluyveromyces marxianus [3]

1. K. marxianus cells (5 g, wet weight) were suspended in 250 mL of milk (Kumamoto Dairy, Kumamoto, Japan)
2. This was mixed with 280 g of commercially available bread flour (Nisshin Seifun, Tokyo, Japan), 4 g of salt (The Salt Industry Center of Japan, Tokyo, Japan) and 7 g of unsalted butter (Megmilk Snow Brand, Co., Ltd., Tokyo, Japan).
3. Bread making was carried out using a bread maker HBK-101 (MK Seiko Co., Ltd., Nagano, Japan) in the natural yeast mode according to the attached manual. Three pieces of the dough were independently baked.

Bread making process for commercial yeast [3]

1. Commercial dry yeast (Nisshin Seifun, Tokyo, Japan). Dry yeast (2.4 g) and 190 mL of tap water mixed with 280 g of bread flour, 4 g of salt, 20 g of unsalted butter and 6 g of skim milk (Megmilk Snow Brand), and 20 g of sugar (Dai-Nippon Meiji Sugar Co., Ltd., Tokyo) were used.
2. Bread making was carried out using a bread maker HBK-101 in the normal mode following the manual attached to the machine. Three pieces of the typical bread were independently baked.

The commercial yeast bread was the same in height, weight, and appearance as the K. Marxianus bread. The use of milk containing moisture and lactose showed no effect on bread properties in comparison to using water and sucrose. The hardness and cohesiveness were not significantly different between the two types of bread. Sensory values between the K. Marxianus and commercial yeast were not significantly different. A test bread made without salt or fat was found to be inferior in terms of the expected flavor. [3]

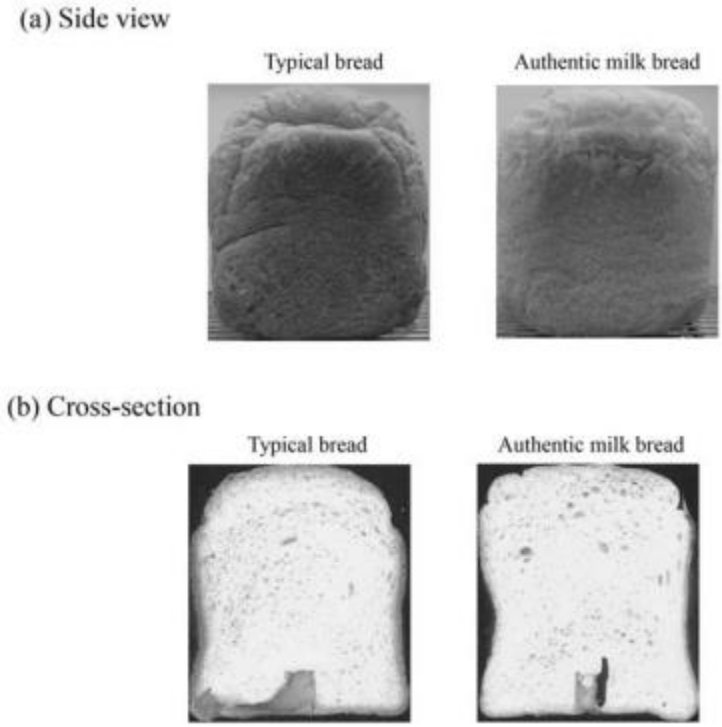

Figure 1. The side view (a) and cross section (b) of typical bread made with commercial yeast and authentic milk bread made with K. Marxianus. [3]

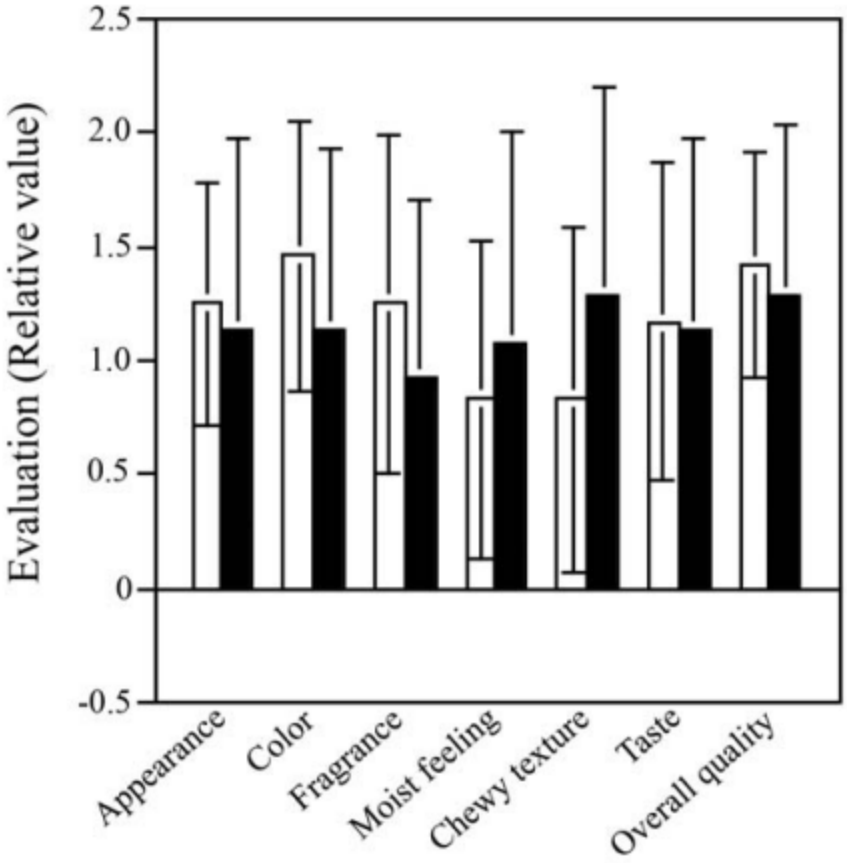

Figure 2. Sensory evaluation of commercial yeast bread versus K. Marxianus bread. Twenty-three volunteer tasters evaluated pieces of bread and gave evaluation as follows. -2 (bad) -1 (slightly bad) 0 (neutral) +1 (slightly good) +2 (good). The error bars indicate stand deviation. Commercial yeast is open bars. Black bars are K. Marxianus bread. [3]

Kluyveromyces marxianus bioprocessing of wheat bran for better bread

Kluyveromyces marxianus produces several enzymes that are active in breaking down components associated with cellulose such as xylanase, endoglucanase, exoglucanase and beta glucosidase. These enzymes are used to bioprocess wheat bran into a more digestible form for human consumption. Wheat bran has many valuable elements for human nutrition such as dietary fiber, vitamins, and minerals. However, the fiber content weakens the gluten network and decreases bread volume and elasticity. Wheat bran also has an adverse effect on flavor and texture of the resulting bread. Arabinoxylan is one factor that affects the gluten

structure. Xylanases solubilize and change the molecular weight of Arabinoxylan therefore altering the water migration in the bread dough. [4]

Materials used [4]

1. Wheat bran (WB) (TDF: 46.4%; protein: 13.9%; ash: 4.1%; fat: 2.4% and starch: 20.2% on dry basis, respectively) was obtained from FZY Flour Co. Ltd (Xingji, China)
2. Xylanase (885,000 U/g) was provided by DSM Co. Ltd (Shanghai, China)
3. Kluyveromyces marxianus (ATCC36534) was purchased from Yi Yan Co. Ltd (Shanghai, China)

Culturing Conditions [4]

1. Kluyveromyces marxianus (ATCC36534) was stored at −80°C and periodically refreshed.
2. Before use, the yeast was revived by culturing in Yeast Malt (YM) broth and incubated at 30°C for 24 hr.
3. The activated K. marxianus was centrifuged at 4,000 g (10 min, 4°C), and the pellet was washed twice with sterile saline and suspended in sterile saline to a concentration of 4 × 107 CFU/ml
4. 100 g raw WB was mixed with 12 ml of yeast suspension and distilled water (Dough yield = 190) and fermented for 48 hr at 30°C.

Fermented Wheat Bran Bread Preparation [4]

1. Four doughs, BB: 80% wheat flour and 20% raw Wheat Bran WB, Fermented Bran Base FBB: 80% wheat flour and 20% K. marxianus pre-fermented wheat bran, Enzyme Bran Base EBB: 80% wheat flour, 20% raw WB, and xylanase, Fermented and Enzyme treated Bran Base FEBB: 80% wheat flour, 20% K. marxianus pre-fermented wheat bran, and xylanase, were prepared.
2. The dry ingredients (240 grams of wheat flour, 60 grams of wheat gran or 114 grams of fermented wheat bran) and water were initially weighed and mixed (SM-25; Sinmag Machinery Co., China) at low speed for 3 minutes and at high speed for 2 minutes.
3. Butter was then added and mixed again for 2 minutes at low speed and 3 minutes at high speed to obtain a smooth dough. 2 ml xylanase solution (90 U/ml) was added directly along with water in EBB and FEBB

4. Furthermore, 2 ml xylanase solution (90 U/ml) was added directly along with water in EBB and FEBB
5. For BB and EBB, dry raw bran was added, whereas in FBB and FEBB, wet and pre-fermented by K. marxianus bran was used wet after pre-fermentation. Water added to dough was adjusted accordingly.

Table 2. The influence of pre-fermented wheat bran and xylanase on bread properties. [4]

Sample code	BB	FBB	EBB	FEBB
Apparent stickiness	20.45 ± 0.48 a	22.78 ± 0.55 b	25.29 ± 0.23 c	22.21 ± 1.10 b
Dough mixing behavior				
Absorption (%)	62.00 ± 1.45 c	58.50 ± 1.58 b	57.70 ± 2.05 a	58.00 ± 0.96 a
Dough development (min)	4.70 ± 0.05 b	6.87 ± 0.02 c	4.64 ± 0.11 b	6.84 ± 0.20 c
Stability (min)	7.07 ± 0.27 b	10.24 ± 0.17 d	6.90 ± 0.09 a	10.19 ± 0.01 d
Fermentation properties				
Development height (mm)	57.50 ± 2.35 a	71.07 ± 2.86 c	60.90 ± 2.20 b	72.87 ± 1.97 d
Retention coefficient (%)	66.10 ± 3.96 a	78.51 ± 2.47 c	73.13 ± 3.46 b	78.60 ± 3.30 c
Bread qualities				
Specific volume (ml/g)	4.49 ± 0.04 a	4.97 ± 0.07 c	4.62 ± 0.03 b	5.26 ± 0.10 d
Firmness (g)	460.67 ± 9.29 d	324.33 ± 8.15 b	395.67 ± 2.08 c	267.67 ± 12.76 a

Notes. BB: 80% wheat flour and 20% raw bran; EBB: 80% wheat flour, 20% raw bran and xylanase; FBB: 80% wheat flour and 20% prefermented bran; FEBB: 80% wheat flour, 20% prefermented bran, and xylanase.
Means ± standard deviation ($n = 3$), different superscripts in the same row indicate significant differences at $p \leq 0.05$.

Table 3. The dietary fiber, arabinoxylan, phenolic acids and acidity of wheat bran and bread formulations. [4]

Sample code	WB	FWB	BB	FBB	EBB	FEBB
TDF (g/100 g)	45.31 ± 0.21 d	40.71 ± 0.78 c	14.51 ± 1.23 b	12.95 ± 2.24 a	14.61 ± 1.89 b	14.36 ± 0.76 b
SDF (g/100 g)	4.25 ± 0.09 bc	7.56 ± 0.08 d	2.48 ± 0.02 a	4.12 ± 0.10 b	2.67 ± 0.06 a	4.37 ± 0.06 c
Total AX (mg/g)	90.63 ± 2.45 d	90.08 ± 3.06 c	34.38 ± 0.91 b	33.77 ± 0.87 ab	32.36 ± 1.21 a	31.87 ± 1.38 a
WEAX (mg/g)	7.01 ± 0.98 b	25.89 ± 1.24 e	6.89 ± 0.87 a	8.62 ± 1.02 c	8.66 ± 1.00 c	9.73 ± 1.06 d
TPC (mg/g)	1,610 ± 23.00 c	2,780 ± 25.76 f	808 ± 10.29 a	1,815 ± 25.90 d	846 ± 11.20 b	1,913 ± 10.02 e
FA (μg/g)	9.67 ± 0.98 b	32.91 ± 1.23 d	2.47 ± 0.09 a	10.06 ± 0.17 b	3.21 ± 0.04 a	12.28 ± 0.35 c
pH	6.27 ± 0.09 a	6.11 ± 0.08 a	6.23 ± 0.02 a	6.17 ± 0.01 a	6.10 ± 0.01 a	6.13 ± 0.04 a
TTA	7.70 ± 0.08 a	14.03 ± 0.19 d	7.73 ± 0.12 a	12.07 ± 0.17 c	8.38 ± 0.16 b	12.35 ± 0.11 c

Notes. AX: arabinoxylan; BB: bran bread, 80% wheat flour and 20% raw bran; EBB: enzyme bran bread, 80% wheat flour, 20% raw bran and xylanase; FA: ferulic acid; FBB: prefermented bran bread, 80% wheat flour and 20% prefermented bran; FEBB: prefermented bran bread with enzyme, 80% wheat flour, 20% prefermented bran, and xylanases; FWB: prefermented wheat bran; SDF: soluble dietary fiber; TDF: total dietary fiber; TPC: total phenol content; TTA: total titratable acid; WB: wheat bran; WEAX: water-extractable arabinoxylan.
Means ± standard deviation ($n = 3$), different superscripts in the same row indicate significant differences at $p \leq 0.05$.

The Effect of Treatments on Dough Properties [4]

1. Dough stickiness increased when enzyme dosage was elevated. With 60 U/100g xylanase, based on flour weight basis, had an equivalent water extractable arabinoxylan as the fermented bran. The fermented bran had higher stickiness than the xylanase applied dough.
2. The addition of wheat bran increases dough adsorption and decreases dough development and stability.
3. Pre-fermented bran and xylanase were similar to each other. Both treatments decreased dough adsorption.
4. Xylanase treatment had a negative effect on dough stability but no discernable effect on dough development.
5. Pre-fermented bran and FEBB increased dough stability as dough development time increased.
6. The increase in dough stability is: Pre-fermented bran (16.70) FEBB (19.66%)

Summary

In this article it has been shown that Kluyveromyces marxianus can be grown in deproteinized whey permeate as a protein rich biomass for a possible alternative to soybean meal. K. Marxianus can also be used as a suitable alternative to commercial yeast when making bread without any decrease in quality parameters. Lastly a method to convert wheat bran into a more digestible form that can improve the nutritional value of bread has also been demonstrated. In the future K. Marxianus may be produced locally and used as an ingredient in Ready to Use Supplemental Foods. This will provide another tool to be used in the management of malnutrition.

References

1. Osendarp, Saskia, et al. "Ready-to-use foods for management of moderate acute malnutrition: considerations for scaling up production and use in programs." *Food and nutrition bulletin* 36.1_suppl1 (2015): S59-S64.
2. Schultz, Nadja, et al. "Microbial production of single-cell protein from deproteinized whey concentrates." *Applied microbiology and biotechnology* 69.5 (2006): 515-520.

3. Shibata, Aya, et al. "Production and evaluation of the authentic milk bread using a lactose-utilizing yeast, Kluyveromyces marxianus." *Biotechnology* 11 (2020).
4. Zhang, Binle, et al. "Use of Kluyveromyces marxianus prefermented wheat bran as a source of enzyme mixture to improve dough performance and bread biochemical properties." *Cereal Chemistry* 96.1 (2019): 142-153.