

Review

Processing, Characteristics and Composition of *Umqombothi* (a South African Traditional Beer)

Edwin Hlangwani ¹, Janet Adeyinka Adebisi ¹, Wesley Doorsamy ²
and Oluwafemi Ayodeji Adebo ^{1,*}

¹ Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg Doornfontein Campus, P.O. Box 17011, Gauteng 2028, South Africa; eddnathan.muk@gmail.com (E.H.); janetaadex@gmail.com (J.A.A.)

² Institute for Intelligent Systems, University of Johannesburg Doornfontein Campus, P.O. Box 17011, Gauteng 2028, South Africa; wdoorsamy@uj.ac.za

* Correspondence: oadebo@uj.ac.za; Tel.: +27-115-596-261

Received: 18 September 2020; Accepted: 10 November 2020; Published: 13 November 2020



Abstract: Traditional beers, such as palm wine, kombucha and others, are notable beverages consumed all over the globe. Such beverages historically contribute to food security on a global scale. *Umqombothi* is a South African traditional beer nutritionally packed with minerals, amino acids, B-group vitamins and much-needed calories. As a result, the production and consumption of this traditional beverage has been an integral part of South African's social, economic and cultural prosperity. Unfortunately, difficulties in bioprocessing operations have limited its availability to household and small-scale production. It is at these micro-production scales that poor hygiene practices and the use of hazardous additives and contaminated raw materials continue to increase, posing serious health risks to the unassuming consumer. This study provides an overview of the processing steps and underlying techniques involved in the production of *umqombothi*, while highlighting the challenges as well as future developments needed to further improve its quality and global competitiveness with other alcoholic products.

Keywords: *umqombothi*; South Africa; bioprocessing; artificial intelligence; health; safety; local beer

1. Introduction

The production of cereal-based beers in Africa date back to 3500 BC [1–3]. Historians believe that the domestication of cereal crops in North Africa, between 4650 and 4350 BC, catalysed interests in beer brewing [4]. Across the continent, millet, maize and sorghum beers are produced in different regions based on the type of crop widely distributed [5,6]. Although sorghum accounts for only 23% of the farmed land area, sorghum beers, usually termed “African opaque beers”, are amongst the most popular types of beer in many countries such as South Africa, Nigeria, Ghana, Togo and Zimbabwe [1–3,5,7].

This diversity in demographics of the African continent has led to variations in respective brewing (production) processes [8]. For example, home-brewed traditional beers in Burundi are mainly made of banana and cereal grains, with names such as *impeke*, *urwatwa*, *kanyanga*, and *isongo* [2]. In contrast, *doro* is a Zimbabwean variation produced from sorghum, sprouted maize and finger millet malt [2,9]. As a result of these variations and locality of the produce, traditional beers are known by their local names: *amgba* (Cameroon), *bili bili* (Chad), *mtama* (Tanzania), *dolo* (Burkina Faso), *tchakpalo* (Ivory Coast, Togo and Benin), *ikagage* (Rwanda), *chibuku* (Zimbabwe), *merissa* (Sudan), *ontaku* (Namibia) and *pito* (Ghana and Nigeria) [1,8].

Umqombothi is an IsiZulu term describing a sorghum-based beer with an opaque pinkish colour, creamy constituency and sour aroma (Figure 1) [2,10]. The consumption of *umqombothi* is common in

religious ceremonies; African festivals; and rituals such as circumcision and initiation school graduation celebrations, communication with ancestors (*amadlozi*), praying for rain, weddings and the handing over of a dowry as well as births and funerals. Thus, similar to other African traditional beers, *umqombothi* forms an important aspect of the cultural, spiritual and socioeconomic activities in the continent [1,2,8]. Not only is this a significant food product, but the famous African singer Yvonne Chaka Chaka had a pop song with “*Umqombothi*” as its title [11], which gained popularity in the early 1980s. In an era where health is an important factor for food-purchasing decisions, *umqombothi* has also been suggested as a healthy alternative to “clear” or western beers [2].



Figure 1. *Umqombothi* (South African traditional beer) served in a cup.

Regardless of the health benefits and affordability, brewing *umqombothi* has not been formally standardised, especially on a household level [1,8]. This may be because the old-age method of making traditional beer requires the knowledge, skill and expertise of the local people, usually African elderly women who use diverse self-taught beer preparation methods [1,2,12]. The lack of standard procedures and guidelines lead to day-to-day fermentation variances which subsequently result in inconsistent taste, body and texture of the beer [3,8]. On a commercial scale, the production is limited to a few local products [8]. Brewers report that the production activities and the monetary reward are often disproportionately unattractive to investors interested in product development and commercialisation [2,13].

Concerns over general hygiene practices, toxic substances, the behaviour, knowledge and attitude of informal brewers and the beer’s microbial composition have equally been raised by South African authorities [2,12]. Contaminated beer has led authorities to close down local pubs (*shebeens*) and publicly dispose home-brewed beer to discourage its consumption [2,12]. A study by Lues [2] found that “50% of brewers washed the containers when visibly dirty, while 45% washed them after use.” A similar finding relating to washing of hands found that 45% of brewers washed their hands only after visiting the bathroom, while 55% washed their hands only when “really” dirty [12]. Unwashed utensils and unwashed vessels largely contribute to the proliferation of spoilage microorganism and unwanted contaminants [2,10,12,14].

In some instances, brewers have added hazardous “ingredients” such as battery ash, battery acid and methylated spirits to make *umqombothi* more concentrated or to superstitiously attract more customers [2,12,15]. Such breaches in household production of the beer are not uncommon, especially where the demand may dictate the production process. During periods of high demand, the cooking step may be skipped and the fermentation process may be shorted and/or halted altogether to

satisfy consumer demand [2,10,12]. As a result, the final beer product is often less nutritious, tasty and safe for consumers [2,3,8,12]. These factors, together with low ethanol content, shorter shelf life and organoleptic variations, have rendered traditional African beer to appear less attractive than barley-brewed western beers [8,16].

The challenges around general quality and the health hazards linked to production and consumption of traditional beer will therefore continue to persist unless adequate efforts are put in place to improve the process and ensure consistency in its composition. This review provides an overview of *umqombothi* production as well as its composition. Within the scope of the production, the underlying science governing each of the processing steps are covered. Challenges of *umqombothi* processing were discussed as well as an appraisal from available literature and information regarding its microbiota and beneficial properties. Attempts at improving its quality were also highlighted with a section of pertinent areas for innovative approaches requiring further development.

2. *Umqombothi*: Its Origins and Processing

2.1. The Socioeconomic, Cultural and Spiritual Significance of *Umqombothi*

Umqombothi is an affordable beverage in low-income households, especially rural areas where poverty and malnutrition are a constant concern [2,12,13]. Low-income populations in semi-urban areas also benefit from consuming this low-cost beer [2,13]. As a result, daily consumption increases as brewers in rural and semi-urban areas prepare and sell *umqombothi* to make a living [10,13]. High financial gains by brewers can thus be expected because of increased consumption and customer demand [1,2,12]. In Dikgale, Limpopo province, South Africa, an average of 1.3 L daily consumption of *umqombothi* per person is reported [13].

Brewers in the Kimberly, in the Northern Cape Province of South Africa, reported an average monthly income of USD 105, depending on customer availability. Conversely, brewers in Bushbuckridge, South Africa, reported earning as little as USD 8 and as much as USD 211 per month [2,17]. In the past, *umqombothi* was purposely brewed for social activities with minimal regard for deriving income [12]. Presently, only 25% is attributed to brewing for social and cultural activities [2]. *Umqombothi* is always a choice of drink during cooperative work, weddings and the handing over of a dowry, circumcision and initiation school graduation celebrations, births and funerals, communication with ancestors or spirits, praying for rain and social meetings [1,2,8,12,13].

2.2. Processing Steps Involved in Brewing Practices of *Umqombothi*

The ingredients used in the making of *umqombothi* are malted cereals (wheat, maize, sorghum), sugar and brown bread (optional), which are broken down by wild yeasts and bacteria to produce an alcoholic end product through a stepwise process, as depicted in Figure 2. On a household level, *umqombothi* is generally prepared by self-taught elderly women by hand-mixing 1 kg wheat malt, 1.4 kg brown bread (optional), 1 kg sorghum malt, 1 kg maize meal, 10 g wet (compressed) yeast, and 1 kg brown sugar in an iron-cast pot or plastic bucket filled with 20 L lukewarm water [1,2,12,13]. Pre-weighed packets of these ingredients are commercially available under different brand names, but these ingredients (if not pre-packed) are seldom weighed [12,18,19]. The elderly women and sometimes men, use previous experience and basically “gauge” the weights by hands, cups or bowls. Thus, like the production of other sorghum beers, the production of *umqombothi* lacks measurement precision necessary for satisfactory quality control [16]. Irrespective of the ingredient source, the mixing process aids the uniform distribution of the raw materials and is usually done in an earthen vessel or iron-cast pot [2,12,13].



Figure 2. *Ukhamba*—a traditional beer-drinking vessel.

Ideally, a previously used iron-cast pot or plastic bucket (sometimes plastic drum) is used to soak the mixed ingredients overnight to foster spontaneous wild-yeasts fermentation, followed by cooking to break up the yeast cells to release locked-up nutrients and gelatinise the starch [1,2,12,13]. While the cooking step is vital, prolonged cooking periods could reduce the nutritional value of the beverage and significantly reduce available sugars for fermentation. Subsequent to this is cooling, conducted in a plastic bucket or iron-cast pot which is then tightly covered overnight to facilitate secondary fermentation, predominately performed by lactic acid bacteria (LAB) [1,2,8,12,14]. During winter time, the container may be covered with a blanket or a fire may be lit to provide a warm fermentation environment favourable for the fermentation microbes. At present, the fermentation of *umqombothi* is allowed to proceed in plastic drums or iron pots [13]. A four-day summary of the traditional method of making *umqombothi* is shown in Table 1.

Table 1. Summary of the household method of making *umqombothi*.

Day	Ingredients	Utensil(s)	Procedure
1	15 L lukewarm water, 1 kg sorghum malt, 1 kg mealie meal, and 1 kg wheat malt	20 L bucket, blanket (optional)	Mix ingredients together in a 20 L bucket. Place lid on top without sealing it and allow fermentation to proceed overnight in a warm area or covered with a blanket and/or placed next to a fire (in winter times). Resuspended ingredients by stirring. Fill a large pot with between 1/5 and 3/5 of water and heat to boiling point. Add the fermented mixture to the boiling water until a slurry is formed. The cooked slurry is left on a large container and allowed to cool throughout the rest of the day and overnight.
2	-	Large container, iron-cast pot	A sufficient amount (1–3 kg) of wheat malt is added to the porridge. The mixture is gently stirred and allowed to ferment overnight in a warm area or covered with a blanket and/or placed next to a fire (in winter times).
3	Wheat malt and previously cooked slurry	Blanket (optional)	The fermented mixture is stirred and the beer strained using a woven sieve (<i>ivovo</i>) to remove unwanted suspended solids. The desired beer can then be served on <i>ukhamba</i> and stored in large plastic drum (<i>gogogo</i>).
4	-	Woven sieve (<i>ivovo</i>), a traditional beer-drinking vessel (<i>ukhamba</i>)	

Formerly, the cooking and fermentation processes occurred solely in iron-cast pots [13]. Iron-cast pots and non-galvanised iron vessels, however, are still vessels of choice for the cooking stage of the process [1,2,13,20]. The beer is then poured into a large plastic drum known as a *gogogo* for sharing with friends and family [2,12,13,21]. Modern brewers use cheap, easy to clean and rust-resistant twenty litres buckets to store the indigenous beer as opposed to traditional clay pots [2,12]. Traditionally, *umqombothi* was served in clay beer-drinking vessel known as *ukhamba* (Figure 2).

During the fermentation process, the fermenting microorganisms produce substances that give *umqombothi* its characteristics pH, aroma, consistency and unique sensory properties. As observed in the literature, different fermentation regimes are used for *umqombothi* (Table 2), and the differences which might have an effect on the overall composition of the product were reported [1,8–10,12,14,16,21–24].

Table 2. Available literature of processing conditions for *umqombothi*.

Fermentation Time (h)	Fermentation Temperature (°C)	Mash Cooking Time (h)	Mash Cooking Temperature (°C)	Secondary Fermentation Time (h)	Secondary Fermentation Temperature (°C)	Reference
72	25–30	3–5	NR	120–168	25–30	[9]
10–12	RT	3	NR	12–24	25	[8]
8	28–29	2.5	106	NR	NR	[1]
24	25–30	NR	NR	24–48	25–30	[12]
24	NR	NR	NR	18	NR	[10]
14	30	0.3	90	NR	4	[24]
12–48	NR	0.8	NR	24	NR	[21]
24–72	NR	4	NR	24–72	NR	[22]
24	NR	2	NR	24	50	[14]
18–20	48–50	1.5–2	50–60	25	25	[23]
NR	48–50	1.5–3	98	25	25	[16]

NR—not reported; RT—room temperature.

2.3. Early Industrialisation Attempts for *Umqombothi* Processing

Early industrialisation attempts were made for processing *umqombothi* in South Africa, with the first commercialisation of *umqombothi* starting in 1908 after the municipal monopoly by town authorities was authorised by Natal legislative assembly in the British colony [25]. Unfortunately, political unrest of the 1970s, the introduction and superior marketing of clear malt beers by South African Breweries (SAB), and consumer repulsion of sorghum beer due to its associations with apartheid government schemes led to the decommercialisation of *umqombothi* from 1980 [25]. To keep up with new consumption trends, private brewing companies such as Ukhamba Beerworx and United National Breweries are resuscitating the industrialisation of *umqombothi* [26,27].

For the industrialised processes, mixing, incubation, boiling, cooling, fermentation and filtration constitute the brewing process of traditional beers [2,12]. Although variations in the brand used exist, 10 kg maize meal and 10 kg maize malt are mixed into a 500 L bioreactor [28,29]. To make a paste (or wort), 2.5 kg wheat malt is added to 200 L lukewarm water (35 °C–45 °C) containing 10 kg maize meal and 10 kg maize malt [23,28,29]. To homogenise and make a “porridge”, the slurry is boiled for 30 min at 45 °C while gently stirring.

Thereafter, the cooked porridge is cooled to an ambient temperature. An additional 7.5 kg wheat malt is added to the cool porridge and homogenised with gentle stirring [16,28–30]. Fermentation with gentle stirring is then allowed to proceed for 8 h [16,29,31]. Depending on the maturation of the brew, the mixture is allowed to ferment for an additional 8 h at 35 °C [12,23,28,29]. The beer is then filtered to remove unwanted or residual suspended solids, and the final product is recovered as depicted in Figure 3 [2,12,32]. The final beer product is often poured into carton boxes (Figure 4), although plastic and glass bottles are also used.

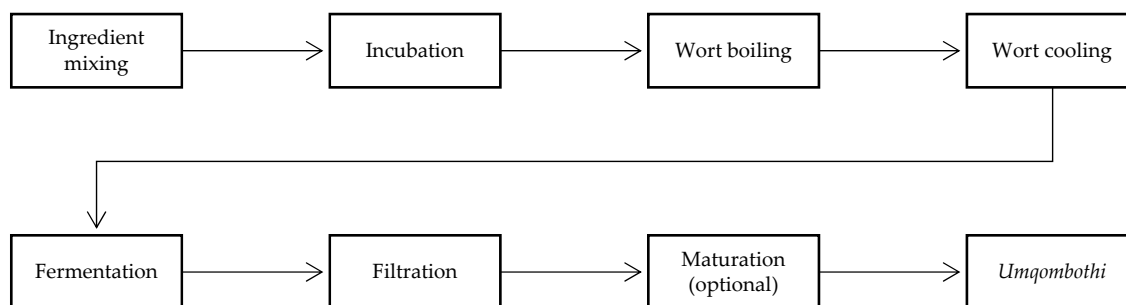


Figure 3. Brewing process of *umqombothi*.



Figure 4. United National Breweries' carton-packaged traditional beer.

3. Composition of *Umqombothi* and Benefits

The composition (functional qualitative aspects) of any type of beer determines its popularity amongst consumers [3,33]. The nutritional, medicinal and sensory properties of beer give it its desirable qualities [3]. In general, sensory, physical and physicochemical, chemical and biochemical and microbiological evaluation are fundamental subcategories of qualitative aspects in beer brewing [3]. As a result, the composition of beer characterises its main qualities: mouthfeel, aroma, appearance and flavour [33]. The type of raw material used as well as the fermentation process are primarily responsible for these sensory qualities [2,8,14,33]. Unfortunately, qualitative aspects are seldom controlled in traditionally manufactured African beers [34]. In addition, there is scarcity of data describing the nutritional and biochemical composition of local sorghum beers [12,34].

3.1. Physicochemical Constituents

Since consumers believe drinking coloured beers is healthy, *umqombothi* is often brewed from pigmented sorghum varieties [8]. The sorghum and ancillary ingredients used dictate the colour, which varies from pinkish brown to a pale buff [8]. This practice means that *umqombothi* is opaque and not clear due to suspended solids. Suspended solids (5–7%) are mostly starch residues, dextrins and dietary fibres, which also give the beer its high calories [1,8,12,20,35]. Depending on where and how the beer is brewed, *umqombothi* has an average pH range of 3 to 4.2, a lactic acid level of 0.26% and a low alcohol content of 2–3.5% *v/v* [8,12,14].

Typically, *umqombothi* has a creamy constituency, distinctive sour taste, and fruity odour [1,2,8,10,12,14]. The sour taste is attributed to presence of LAB and formation of organic acids during fermentation [2,12]. The fruity odour may occur as a result of the presence of certain microorganisms, especially *Candida* species and/or the production of diacetyl from wild-type strains of *Saccharomyces* species [1,8]. Traditional beers, including *umqombothi*, have a short shelf life (24 h–72 h) and are thus consumed in an actively fermenting state [1,8,10,14].

3.2. Nutritional Content and Health Benefits

Umqombothi is a calorie-rich beverage able to provide 130–394 kJ/100 g (Table 3), making it a great source of energy for millions of African people, especially in poverty-stricken societies [1,8,20]. The raw materials and the fermentation process involved makes *umqombothi* rich in B-group vitamins including thiamine (B₁), riboflavin (B₂), niacin (B₃), pantothenic acid (B₅), pyridoxin (B₆), folic acid (B₉) and vitamin C (Table 3) [1,2,8,12,14]. These vitamins are responsible for genomic and non-genomic methylation, clearance of homocysteine, the synthesis of numerous neurochemical transmitters (such as dopaminergic and serotonergic neurotransmitters) and signalling molecules, energy production, DNA/RNA synthesis or repair, and cellular function [36,37]. Vitamins are thus fundamental for longevity, good brain function and general health [36,37].

In addition, *umqombothi* has a wide variety of essential amino acids (EAAs): leucine, lysine, phenylalanine, tryptophan, and valine, as well as non-essential amino acids (NEAAs): aspartic acid glutamic acid and alanine [1,2,8,12,14]. *Umqombothi* is particularly rich in leucine (Table 3), an amino acid essential for ATP generation and protein synthesis [27,38–40]. Collectively, EAAs and NEAAs induce apoptosis in cancer cells, increase lean muscle mass, promote mitochondrial biogenesis, enhance fertility, prevent liver damage, improve metabolic profiles, promote collagen integrity, prevent insomnia, accelerate wound healing and improve mortality. Furthermore, these amino acids participate in cell signalling, pigmentation, regulation of gene expression, regulation of immune function, removal of toxic substances, regulation of endocrine status and affect the digestive and absorptive function of the small intestine [30,38–42].

The presence of probiotics and prebiotics (dietary fibres) in *umqombothi* promotes good gut health, favourable bowel movements and antimicrobial properties [5,43]. LABs are the dominant microorganisms involved in the fermentation of *umqombothi* and are thus beneficial to the fermentation process and its end product [1,7,8,43]. Rural communities have derived health benefits through the consumption of probiotic rich-fermented beverages such as *umqombothi* [7,43]. These probiotics are known to administer bactericidal, bacteriostatic and antimicrobial effects and thus improve intestinal microbial balance of the human body [7,43]. In addition, probiotics promote overall health by optimising gut ecology, antagonising pathogens and spoilage bacteria, improving nutrition modulating the body's immune response, and soothing intestinal disorders [43].

Table 3. The nutritional composition of *umqombothi*.

Nutritional Parameters	Nutrient per 100 g	Reference
Calories (kJ)	130.12–394	[8,27,34,44]
Dietary fibre (g)	5–14.5	[45]
Protein (g)	0.5–8.7	[8,27,34]
Carbohydrate (g)	3.6–4.8	[8,34]
Thiamine—B ₁ (mg)	0.1–3.4	[8,27]
Riboflavin—B ₂ (mg)	0.05–0.76	[8,27]
Niacin—B ₃ (mg)	0.008–0.4	[8,27]
Pantothenic acid (B ₅) (mg)	0.09	[8]
Pyridoxin—B ₆ (mg)	0.17–0.59	[7]
Folic acid—B ₉ (mg)	0.2	[7]
Cyanocobalamin—B ₁₂ (µg)	0.03	[8]
Vitamin C (mg)	0.04	[8]
Leucine (g)	11.74–14.8	[7]
Lysine (g)	7.2	[27]
Phenylalanine (g)	4.03–5.62	[7]
Tryptophan (g)	0.9–1.16	[7]
Valine (g)	4.22–6.86	[7]
Aspartic acid (g)	4.83–7.06	[7]
Glutamic acid (g)	17.50–28.12	[7]
Alanine (g)	7.34–9.62	[7]
Calcium (mg)	2.2–20.7	[8,27]
Magnesium (mg)	144	[45]
Zinc (mg)	2.84	[45]
Sodium (mg)	1.1–26.7	[8,27]
Manganese (mg)	1.91	[45]
Potassium (mg)	84–1101	[8,27]
Phosphorus (mg)	39	[8]
Iron (mg)	2.55–6.08	[8,20,45]

g—gram; kJ—kilojoule; mg—milligram.

van Heerden published two studies in the late 1980s determining the dietary content in sorghum beer strainings and sorghum beers brewed with sorghum adjunct to be 50 g/kg and 1.1 g/L [44,45]. Typical modern-day sorghum beers contain about 14.5 g/100 g of dietary fibre, which is sufficient for the recommended daily intake of 14 g/1000 kcal for children and adults (Table 3) [27,46]. The size of the sorghum grain will dictate the prebiotic content, which can range from 2% to 30%, with smaller grains having a higher proportion of husk [27]. The fermentation of some of these dietary fibres can lead to the generation of bioactive compounds such as short-chain fatty acids (SCFAs), which transform the gut's microflora by increasing its biomass [47,48]. As a result, regular consumption of prebiotics reduces risk of developing type-2 diabetes, colon cancer, obesity, haemorrhoids, hypertension, gastroesophageal reflux disease, diverticulitis, coronary heart disease, stroke, and duodenal ulcers [46–48]. Diabetic and non-diabetic individuals can benefit from lower serum cholesterol levels, weight loss, glycemia and insulin sensitivity, enhanced immune function, lipid metabolism, food digestion, and fibre-induced mineral bioavailability [46–48].

In a time where mineral deficiencies are prevalent, *umqombothi* can provide significant amounts of magnesium, zinc, manganese, phosphorus and bioavailable iron (Tables 3 and 4) [1,8,14,20]. Sorghum malt, the main ingredient used in today's preparation of the beer, is rich in manganese, zinc, phosphorus, potassium, copper and iron [7,8,45]. In the case where sorghum grains are used, the germination process increases the availability of minerals, as well as essential amino acids [6]. In addition, minerals are made bioavailable through alcoholic fermentation [27]. A study at Dikgale field site and the surrounding villages in Limpopo Province, South Africa showed that the consumption of *umqombothi* prevented iron deficiency in people at risk [13]. When compared to non-drinkers,

women of childbearing age who consumed the beer had significantly higher concentrations of transferrin and serum ferritin [20].

Table 4. The mineral content in *umqombothi* derived from sorghum adjunct and percentage contribution to the recommend daily allowance (RDA) by a litre of the beer.

	Concentration (mg)	RDA (mg)	% Contribution/L of Beer to RDA
Mg	178	350	51
Mn	1.83	4	46
P	305	800	38
Fe	3.44	10	34
Zn	1.94	15	13
K	407	3760	11
Cu	0.27	3	9
Ca	52	800	7
Na	18	2300	1

Mg—magnesium; Mn—manganese; P—phosphorus; Fe—iron; Zn—Zinc; K—potassium; Cu—copper; Ca—calcium; Na—sodium. Adapted from van Heerden [45].

To conclude, Mandishona et al. [20] added, “the consumption of traditional beer, rich in iron, protects women against iron deficiency.” Generally, these minerals are responsible for cellular regulation and cellular build-up of living cells and can thus aid in fighting mental health disorders such as chronic anxiety and depression [1,49–53]. In interviewing regular consumers of *umqombothi*, Ikalafeng [12] recorded, “the consumers advanced reasons such as stress relief and making their lives easier as some of the factors influencing their drinking habits. It became evident that what was originally a traditionally cultural beverage has taken on a new form, that of a psychological intervention”.

3.3. Microbiota

Household and small-scale industry brewers often employ spontaneous fermentation practices in the production of *umqombothi* [1,2,10,12,13,54]. Since there is generally no addition of exogenous “starter cultures”, lactic acid and alcoholic fermentation are spontaneously carried out by the natural microbial flora present in one of the raw materials, i.e., malted sorghum, malted maize, bread or wheat [1,2,10,12,13,54]. A common and hazardous practice is the use of previously used and unwashed utensils to mix, soak and enable spontaneous fermentation [2,12]. The microcolonies of yeast–bacteria associations as examined by Katongole [14] in biofilm formation on the surface of unwashed pot surfaces revealed a high occurrence of unidentified and potentially harmful yeasts and bacteria.

During the mashing process, where the ingredients are mixed, soaked and left overnight to sour, spontaneous lactic acid fermentation is carried out by wild yeasts and heterofermentative and homofermentative mesophilic LAB, which may pose potential human health risks [1,54–59]. At this stage, lactic acid bacteria are the dominant species [60]. Van Der Walt [54] found *Pediococcus damnosus*, *Lactobacillus delbrueckii*, *Levilactobacillus brevis*, *Leuconostoc dextranicum*, *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides* and *Limosilactobacillus fermentum* to be common isolates in mash as introduced by the ingredients used. Furthermore, the author suggests that the predominance of one species or the other will be determined by the souring temperature, leading to differences in the lactic acid flora profiles in the brew. Specifically, seeding the mash with *Lactobacillus delbrueckii* at 45 °C has the advantage of reducing the hazard of the development of unwanted microbes in the souring mash, reducing the souring time from 12 to 6 h, and producing the laevorotatory isomer of dextrorotatory and racemic lactic acid, which is nutritionally preferable when a highly acidic beverage such as *umqombothi* is consumed [54].

To achieve diastatic conversion in the traditional method, additional water and malted sorghum are often added to the soured mash to spontaneously facilitate alcoholic fermentation [54]. Yeasts such as *Pichia anomala*, *Kloeckera apiculata*, *P. fermentans*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*,

Candida krusei, *Endomycopsis fibuligera* and *C. tropicalis* have been isolated in both the sorghum malted and the beer [54]. Of the three dominant yeasts, *C. krusei*, *K. apiculata* and *S. cerevisiae*, only *S. cerevisiae* is the significant microorganism due to its superior fermentative ability [54,55]. Industrially, it is common practice to pitch with selected strains of *S. cerevisiae* to achieve optimum fermentation [54]. There is no doubt that quality of *umqombothi* can be enhanced, even on small-scale basis, through the use of commercial and/or tailored autochthonous starter cultures, including selected *S. cerevisiae*, non-*Saccharomyces* and LAB [56–59]. In a study where three different strains of *S. cerevisiae* were used as inoculum for an 8-day fermentation period, a 3–28% loss of fumonisin B₁ (FB₁) and a 9–17% loss of fumonisin B₂ (FB₂) was observed [60].

The use of “open” and spontaneous fermentation by independent brewers poses the risk of wild microbial contamination and proliferation, directly impacting the safety and sensory quality of *umqombothi* [12]. Off-flavours are produced when microbes not originally present in the raw materials contaminate the beer [12]. The development of *Acetobacter* species, in particular, promotes the formation of volatile acid, thereby reducing the keeping value of *umqombothi* [54]. In an attempt to achieve diastatic conversion, selected non-toxigenic strains belonging to the genera *Aspergillus*, *Penicillium*, *Mucor*, *Oospora* and *Rhizopus* can be reintroduced into boiled, sterile porridges [54].

The presence of microorganisms in fermented products make them vital for health and longevity on a global scale [60]. It has thus become necessary to assess the ecosystems in which these microorganisms exist and how they interact to provide safe and quality food products [61]. Yeasts such as *Issatchenkia orientalis*, *K. apiculata* and *S. cerevisiae* and bacteria such as *Levilactobacillus brevis*, *Limosilactobacillus fermentum* and *Lactiplantibacillus plantarum* are health-imparting microbes found in *umqombothi* [1–3,8,10,12,54–58].

In ascertaining food microbial ecologies in the modern era, next-generation sequencing (NGS) has proven itself to be a superior tool of microbial identification. As a result, microbial communities in their entirety (i.e., starter cultures and food spoilage microorganisms) and their genetic, metabolic and physiochemical impact on a particular food can be studied [61–64]. Driven by NGS technologies, classical environmental studies and microbial ecology have evolved into a new field of “metagenomics” [65–67], with found application in the food safety assessment and correct management of food fermentations [68–71]. Staley and Sadowsky [67] described metagenomics as the direct genetic analysis of genomes contained within an environmental sample without prior need for cultivating clonal cultures. Metagenomics is a low-cost microbial identification approach able to provide genetic information on potentially novel biocatalysts, evolutionary profiles, genomic linkages between function and phylogeny for uncultured organisms and broad phylogenetic surveys [67].

4. Safety Challenges with *Umqombothi* Processing

Under normal circumstances, traditional beer brewing is a complex bioprocess, making commercialising difficult, especially without the application of innovative solutions [1,8,72]. The production of the traditional beer has been known to take up to five days and is only shortened to three days, depending on consumer demand [2,10,14]. However, this reduction in fermentation time has been associated with bitter, tasteless and poor-quality beer [3,8]. In addition, this practice may promote the proliferation of spoilage microorganisms such as *Escherichia coli*, *Rhizopus stolonifer*, *Penicillium crustosum*, *Staphylococcus aureus*, *Aspergillus parasiticus*, *A. flavus* and *Fusarium verticillioides* [1,10,12,14]. Conversely, essential fermentation microorganisms are destroyed during the process of excessive heating, sieving, and harsh treatments [3,8]. South African authorities have raised concerns about toxic substances, hygiene practices, microbial contamination and unauthorised practices of informal brewers [2,12,14]. In conjunction with the above-mentioned factors, incomplete fermentation, a high percentage of microorganisms and solids, a short shelf-life and organoleptic variations have rendered traditional African beer to appear less attractive than barley-brewed western beers [8,16]. Thus, for traditional beer to be competitive in the global beer market, novel bioprocessing approaches need to be implemented [72].

Ikalafeng [12] analysed South African traditional beer samples in marginal urban settlements in the greater Kimberley area of the Northern Cape Province and found that the mean total viable coliforms and *Staphylococcus* species were both 10^6 CFU/mL and total fungi was 10^7 CFU/mL. In characterising *Staphylococci* in commercially brewed *umqombothi* and home-brewed *umqombothi*, *S. hominis*, *S. epidermidis*, *S. aureus*, *S. saprophyticus* and *S. xylosus* were predominant in both beer types [12]. The high total fungal counts may be attributed to a yeast-favourable pH and yeast-favourable raw materials such as sorghum, maize and bread [7,12]. Katongole, [14] observed that the occurrence of moulds decreased by log 1 to non-detectable levels after soaking the sorghum and maize mixture. Faecal contamination, indicated by the high prevalence of total coliforms and *Staphylococci*, possibly resulted from poor hygiene practices [12]. Similarly, *Enterobacteriaceae* counts in home-brewed *umqombothi* increased after mash cooking, which was attributed to handler-prone contamination [14].

As a result of poor farming, handling, processing and storage practices across Africa, home-processed traditional beers are often heavily contaminated with several mycotoxins [73,74]. Sporadic reports covering beers prepared using sorghum, maize or millet from South Africa, Nigeria, Zambia, Kenya and Lesotho have shown the presence of zearalenone, fumonisin and aflatoxins [60,73]. In rural communities of South Africa, the dependency on cereal grains as a source of food security reinforces the dietary pattern and ethnic tradition of consuming cereal-based fermented products such as *umqombothi* [2,12,13,57,73,74]. Mycotoxin-related health risks are posed as a result of using highly contaminated home-grown maize in subsequent brews [74]. The harvesters separate the maize harvest by appearance (mouldy and non-mouldy), with the mouldy maize often used as an ingredient in *umqombothi* brewing, as it is believed to impart a desirable taste to the final beer product [60].

In a survey conducted between 2001 and 2004, only 25% of households in Centane (Eastern Cape Province, South Africa) admitted to the practice of using mouldy maize in traditional beer brewing, whereas 50% of households in Bizana (Eastern Cape Province, South Africa) reportedly used mouldy maize in traditional beer brewing [60]. In ascertaining fumonisin exposure estimation in *umqombothi*, calculations based on South African commercial beer consumption data were used due to the lack of consumption data for home-brewed beer [73]. The mean total fumonisins in the beer were found to be 0.2 to 1.0 $\mu\text{g/kg}$ body weight/day and 12–59 $\mu\text{g/person/day}$ [73]. For consumers drinking the beer 2–7 days a week, their fumonisin exposure was 6 times (12.0 $\mu\text{g/kg}$ body weight) higher than provisional maximum tolerable daily intake of 2 $\mu\text{g/kg}$ body weight/day set by the Joint FAO/WHO Expert Committee on Food Additives [73].

An investigation of the mycobiota and co-occurrence of mycotoxins in *umqombothi* by Adekoya [7] revealed a mean fungal load of 3.66×10^5 CFU (colony-forming unit)/mL present in the predominant fungal genera (*Phoma*, *Aspergillus*, *Saccharomyces* and *Penicillium*), with *Aspergillus flavus* having the highest incidence (26%). Furthermore, the study raised concerns about a typical South African's mycotoxin exposure being above the maximum tolerable daily intake of 2 $\mu\text{g/kg}$ body weight/day as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [7,12]. An average 60 kg adult consuming between one and six litres of *umqombothi* a day is exposed to an estimated 2.20–13.20 $\mu\text{g/kg}$ body weight/day exposure of FB₁ and FB₂ [7]. Deoxynivalenol (found in 84% of the samples), enniatin B (found in 75% of the samples) and FB₁ (found in 53% of the samples) are associated with cytotoxicity, carcinogenicity, neural tube defects and hepatotoxicity, as these mycotoxins are highly reactive in humans and animals [7,12,14].

In Centane, Eastern Cape Province, South Africa, the prevalence of fumonisins, especially from cereal grains (i.e., maize, sorghum), has been associated with high rates of oesophageal cancer [60,74]. Upon quantitative investigation of total fumonisins (FB₁ + FB₂ + FB₃) present in traditional *Xhosa* maize beer, FB₁ had the highest incidence (with a mean of 76%) in samples containing all three analogues [60]. The study further found the mean total fumonisin levels in the beer samples from the chosen regions of Centane and Bizana in the Eastern Cape Province to be 502 ng/mL and 284 ng/mL, respectively [60].

It is thus useful to apply a comprehensive management approach to reduce mycotoxin levels in crops often used as raw materials in the production of traditional beers, thereby diminishing

any potential carryovers into the final beer product [73]. To mitigate challenges around mycotoxins occurrence in African crops, Chibundu [73] suggests applying biocontrol technologies to reduce aflatoxins in maize by planting high-quality seeds, carefully sorting cereal grains, timely harvesting and drying crops, and properly transporting and storing crops. In addition, using sorghum or millet as the main ingredients in the processing of novel beverages instead of using maize, which is highly susceptible to aflatoxin-producing *Aspergillus* spp., is recommended [73]. Mycotoxin levels in contaminated raw materials can be reduced by processing steps such as sorting, washing, steeping, dehulling, milling, boiling, roasting and fermentation [73].

An emerging and important concern around *umqombothi* bioprocessing and consumption is the production of toxic biogenic amines from microorganisms used as naturally mixed starter cultures [75,76]. This is because the biogenic amine concentrations are used as an indicator of spoilage in food [75]. When consumed in excessive quantities, biogenic amines can cause adverse health effects [75,76]. For example, many cases of food intoxication are mainly caused by histamine and tyramine [75]. Undesirable effects of histamine may be intensified by other amines such as phenylethylamine, putrescine and cadaverine. When ingested in excessive amounts, histamine is known to cause localised inflammation, rash, diarrhoea, burning and itching, urticaria, vomiting, oedema, palpitations, tingling, nausea and hypotension [76].

Similarly, high levels of tyramine can indirectly cause increased respiration and blood sugar levels, dilation of pupils and swelling of eyes, headache and salivation. Biogenic amines such as agmatine, spermidine, putrescine, spermine and cadaverine can form carcinogenic nitrosamines by reacting with nitrites [76]. In a 2010 study, Magwamba [76] found putrescine to be the most prevalent biogenic amine, and histamine to be the least prevalent in sorghum beer. Furthermore, from the 87 samples analysed for histamine, putrescine, cadaverine and tyramine, 91% contained biogenic amines [76]. A comprehensive analysis of biogenic amines (mg/100 mL) in sorghum beer is shown in Table 5. Similar to findings by Matsheka [75], putrescine was produced in significant amounts (4.04 mg/100 mL) in decarboxylase broth by a *Paenibacillus azotofixans* bacterial isolate (Table 5) [75].

Bacillus cereus, the only food poisoning pathogen isolated from the beer in the study, produced the highest tyramine (3.45 mg/100 mL) and cadaverine (19.8 mg/100 mL) (Table 5) [75]. A lack of good hygiene practices during beer brewing is suggested through the detection of putrescine and cadaverine, biogenic amines associated with poor sanitary conditions [76]. It is likely that the present biogenic amines are formed by decarboxylase-positive microorganisms in the beer [76,77]. The used raw materials used for brewing the beer can also bear endogenic biogenic amines [76].

The formation of carcinogenic nitrosamines in *umqombothi* has been attributed to iron overload, the reaction of biogenic amines with nitrites and fumonisins-producing *Fusarium* fungi [76,78,79]. N-nitrosamines, which can cause oesophageal cancer or squamous cell carcinoma (SCC) of the oesophagus, have been detected in particular brews of *umqombothi* [79]. *Fusarium moniliforme*, a corn saprophyte, is believed to play a role in the production of carcinogenic N-nitrosamines in traditional beer [79]. The annual frequency of SCC rose from the 1930s amongst black South Africans when the staple diet changed from sorghum to maize, with traditional beer being brewed with maize as one of the main ingredients [78,79].

A study in Zimbabwe focused on the consumption of traditional beers closely related to *umqombothi* showed a correlation between regular consumption and iron overload [13]. A similar study in Limpopo, South Africa, attributed the public health problem of iron deficiency and iron overload to consuming locally produced traditional beers [13]. Iron pots often rust with regular use and consistently release iron oxide residues which are deposited in human tissues as hemosiderin when the beer is consumed [79]. Data from Isaacson [79] show that patients with SCC consume more traditional beer than controls. Importantly, however, a lower incidence is observed in African countries in which the staple food is sorghum [78]. It is also vital to highlight that there are general concerns regarding health challenges and toxic effects related to the consumption of alcohol, especially at high levels.

Table 5. Bacterial isolates from sorghum beer (*umqombothi*) and the amounts of biogenic amines each produced in decarboxylase broth.

Bacteria	^a No	BGA Content (mg/100 mL)			
		His	Put	Cad	Tyr
Endospore formers					
<i>Bacillus subtilis</i>	80	1.86	0.10	0.122	0.1
<i>B. thermoglucosidasius</i>	NR	NR	NR	NR	NR
<i>B. cereus</i>	20	1.65	0.26	19.8	3.45
<i>B. laevolacticus</i>	NR	NR	NR	NR	NR
<i>B. halodurans</i>	48	1.05	0.11	0.31	1.772
<i>B. megaterium</i>	NR	NR	NR	NR	NR
<i>B. coagulans</i>	NR	NR	NR	NR	NR
<i>Paenibacillus azotofixans</i>	39	ND	4.04	2.36	1.88
<i>Clostridium</i> spp.	67	1.11	0.23	0.42	1.01
Enterobacteriaceae					
<i>Enterobacter aerogenes</i>	NR	NR	NR	NR	NR
<i>E. intermedius</i>	64	ND	7.50	ND	2.02
<i>C. freundii</i>	NR	NR	NR	NR	NR
<i>C. braakii</i>	NR	NR	NR	NR	NR
<i>Hafnia alvei</i>	NR	NR	NR	NR	NR
<i>Escherichia coli</i>	NR	NR	NR	NR	NR
<i>Pantoea citrea</i>	NR	NR	NR	NR	NR
Pseudomonads					
<i>Pseudomonas fluorescens/putida</i>	NR	NR	NR	NR	NR
<i>P. seudomonas</i> spp.	NR	NR	NR	NR	NR
Staphylococcus					
<i>Staphylococcus epidermis</i>	NR	NR	NR	NR	NR
<i>S. auricularis</i>	NR	NR	NR	NR	NR
<i>S. arlettae</i>	NR	NR	NR	NR	NR
<i>S. aureus</i>	NR	NR	NR	NR	NR
Lactic acid bacteria					
<i>Enterococcus faecium</i>	NR	NR	NR	NR	NR
<i>Ent. faecalis</i>	NR	NR	NR	NR	NR
<i>Ent. gallinarum</i>	NR	NR	NR	NR	NR
<i>Lactobacillus</i> spp.	67	ND	ND	ND	NR
<i>Carnobacterium gallinarum</i>	52	ND	ND	ND	NR
<i>Streptococcus oralis</i>	35	2.34	4.20	3.81	NR
<i>Lactococcus raffinolacticus</i>	29	ND	ND	ND	NR
<i>Pediococcus dextrinicus</i>	23	ND	ND	ND	NR
<i>Leuconostoc mesenteroides</i>	ND	ND	ND	ND	NR

^aNo—for each bacterial strain numbers, two randomly selected strains were tested for the production of a given biogenic amine in decarboxylase broth, and an average was calculated; BGA—biogenic amines; His—histamine; Put—putrescine; Cad—cadaverine; Tyr—tyramine; ND—not detected, NR—not reported. Adapted from Matsheka [75].

Other challenges around the health hazards linked to the production and consumption of traditional beer will continue to persist unless exhaustive scientific investigations are carried out, documented and publicised [1,2,12]. According to Adebo [7], to ensure the safety of fermented foods, less contamination must be ensured in raw materials, whilst ensuring the sterility of processing equipment and all other items during the production of the fermented food. Equally important are hygienic conditions for the handling, packaging and storage of fermented foods in order to mitigate against post-processing contamination, with reported petitions to the government in this regard to provide good hygiene and safe production guidelines to household-level brewers [12,16].

In response, the government has made mention of controlling the production and consumption of home-brewed beer such as *umqombothi*, especially in rural areas [25,80]. Emphasis is placed on having safe and consistently locally produced beverages which can positively contribute to the economy [16,25]. To achieve both ends, bioprocessing approaches can also assist in ensuring efficient production,

consistent composition and safer beer products necessary to stimulate socioeconomic growth, especially in the 21st century [16]. Therefore, the socioeconomic, nutritional, cultural and spiritual significance of *umqombothi* can only be preserved by closely monitoring process parameters and continually improving production through the application of bioprocessing optimisation approaches [16,72,81,82]. The latter is further discussed in the next section.

5. Bioprocessing Approaches

5.1. Classical Bioprocessing Optimisation Approaches

Brewers and production technicians require optimal bioprocessing interventions to ensure a robust control of process and quality parameters, which are both crucial for beer consumers' acceptance [29]. This demand has necessitated the development of novel optimisation techniques and models [29]. Notably, achieving this has been a challenging task, especially in the bioprocessing of beer (both traditional and western) due to biochemical reactions complexities, time constraints and variable interactions within such processes [29,72].

As a result, the relationship between process input variables and the quality of the product cannot always be clearly discernible [72]. Nevertheless, the efficient application of empiric mathematical and statistical models such as response surface methodology (RSM) yields vital information needed to understand, analyse and predict bioprocess responses necessary to correlate and optimise key input parameters that directly affect the product output [29,81,83]. The prediction response is presented in the form of a second-order polynomial equation, as depicted in Equation (1):

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where Y is the response variable (optimal production parameter); β_0 is the intercept of the response variable; and β_i , β_{ii} , and β_{ij} are coefficients corresponding to the factors x_i and x_j ($i, j = 1, 2, \dots, n$). The input variables that affect the response Y are x_i and x_j . The random error is represented by ε [29,81,83].

In bioprocess optimisation, RSM statistically determines multivariate problems from quantitative data [84]. A three-dimensional graphical view with a fitted mathematical model fully describes the concept of RSM [83]. As such, RSM has been heavily applied in food engineering and biotechnology to screen and optimise process factors [29,81,83–86]. In contrast to factorial treatment, RSM-designed experiments provide value predictions of the response variable in relation to the role of independent variables [85].

Although a powerful mathematical tool, RSM is riddled with limitations in determining the linear, interactive and quadratic effects of bioprocess [29,85,86]. In addition, this method increases research labour, time and cost with an increase in the number of experimental runs or input factors [29,81–84]. As a result, machine learning methods such as evolutionary algorithms (EA) and artificial neural networks (ANN) have been applied in conjunction with RSM to ascertain biological interactions within these biological processes [29,51,81–86].

5.2. Bioprocess Optimisation Approaches Using the Concepts of the 4th Industrial Revolution

Artificial intelligence (AI) generates new industrial paradigms by leveraging “enabling technologies”: cloud computing, virtual and augmented reality, advanced embedded systems, big data, internet of things, and cognitive systems [86]. As a result, AI has become an invaluable resource in law, business and economics, education, science and technology, industry and engineering, social studies and agriculture [86,87]. AI is the use of science, technology and engineering to make machines which can emulate human intelligence such as learning, decision, and problem-solving [86]. AI categories can be divided into expert systems, artificial life, data mining, distributed AI, theory of computation, knowledge representation and systems, genetic algorithms, reasoning, machine learning,

constraint satisfaction, natural language understanding, programming, neural networks, belief revision and theorem proving [86].

In bioprocesses involving living cells, active enzymes and metabolites, it is crucial to study, model and optimise the reaction system (i.e., fermentation) to increase the efficiency of the process [88]. Automated online analysis, together with different computer software programs that provide predefined bioprocess optimisation strategies, versatile sample processing, and feed control, is improving the management of complex dynamic biological systems [89]. Other online platforms allow for the *ex vivo* measurement of extracellular and intracellular enzyme activity, while making the quantitative determination of metabolic flux dynamics possible [89,90]. Furthermore, bioprocess development, especially in biotechnology, has experienced the replacement of empirical strategies by new systematic protocols of experimentation, with better reproducibility and analytical precision accuracy [89].

Qualitative models are being replaced by quantitation, which provides meaningful insights into natural systems [89]. As such, the ambiguity circumscribing molecular systems of the natural work can be hurdled by scientific quantitation tools [89]. The demand for integrating computer science in the field of life sciences has revealed new insights into the interaction of food, microbe and mankind [91,92]. An important new-age tool in this development is ANNs, which are currently the most applied and fastest-growing branch of AI [86]. Classical bioprocess optimisation methods can no longer work in isolation since they ignore the combined interaction between experimental conditions of a process by varying one parameter at a time [88].

Thus, the application of ANNs is increasingly becoming popular due to their powerful data-driven computational prowess, their ability to capture non-linear and complex data and their flexibility in analysing biological interactions in their entirety [88,93]. Reved [89] described ANNs as “computer-based problem-solving systems of evolutionary computation field based on the principle of evolution theory.” This technology as a non-linear multivariate modelling tool is being employed by many researchers in food fermentation, production and bioprocess development to better both the process and the product [87,89,94,95]. The main advantage of ANNs, especially for optimising indigenous food fermentation like that of *umqombothi*, is its standard framework’s ability to use background information to solve problems. In addition, ANNs, in contrast to classical tools such as RSM, possess a generic modulation ability (RSM is useful only for logarithmic approaching variables), while not requiring any adequate practical pre-specification [83,89,96].

The superiority of ANNs over RSM as a bioprocessing approach in the fourth industrial revolution lie in their inherent abilities: faultless oversimplification of invisible designs, real-time learning, high parallelism, vigorous elucidation, noise and fault tolerance, data classification and pattern recognition, accurate value predictions, good generalisation capabilities, and knowledge competence for treating erroneous and unclear statistics [82,83,89,96]. A major drawback of ANNs is their use of the black box learning technique, which cannot always correlate input variables and the output [93]. In this instance, RSM becomes useful for better interpretation of the interaction between input and response variables [93]. Thus, to elicit an accurate prediction or response, ANNs must be used in combination with RSM [89,93].

The importance of RSM, despite its limitations, must not be underestimated, since it is still one of the most effective methods improving fermentation media, synthetic or otherwise [88–90]. With a smaller number of variables, RSM can produce a highly accurate response (or output) in non-linear optimisation [90,91]. In addition, RSM can generate a mathematical model in the form of a 3D plot or a second-order polynomial equation, both useful in industrial applications [88,89,97,98]. As a result, it has become a useful tendency to use a coupled bioprocess optimisation approach involving ANNs and RSM in the design of mathematical models and improvement of food production bioprocesses [81–83]. Perhaps in this decade, we will catch a glimpse of the achievement of these tools in accurate bioprocess development, biomathematical modelling, cost reduction and wide application.

6. Future Directions and Conclusions

The importance and relevance of African traditional foods and beverages have gained interest in the last decade [1,6–9]. The health benefits enjoyed by our ancestors in the years gone by are motivating researchers to examine how the same and even better health benefits can be enjoyed today [1,8,20,43,45]. Novel production and bioprocessing methods are being developed to make these foods and their nutrients bioavailable and affordable to produce at a large scale [86–89,97,98]. Moreover, studies are showing interest in understanding the biological interactions that occur within the entire production and supply chain—from farm to consumer—in order to improve quality, safety and sustainability of the food product [73,99,100]. Metagenomics, metabolomics and nutrigenomics are some exhaustive methods of analysis being used to assess the occurrence, expression and consequences of genes, metabolites and nutrients on a host organism [60–67,101]. At the same time, machine learning and artificial intelligent tools such as fuzzy logic, ANNs, particle swarm optimisation, genetic algorithms (GA) and ant algorithms are being applied to understand biological processes as well as generate optimal substitutes for producers. Researchers are examining the aids of technology to enhance traditional methods of production to modern, cost-effective and efficient processes [1,6,54–62].

The development and further commercial production of South African traditional beer was encouraged by author Dr. Van Der Walt's in 1954 with a study titled “Better Kaffir Beer” [55]. Since then, not much has been achieved with respect to scientific enquiry as an attempt to understand the dynamics involved in the production of such a product [73]. As a result, traditional beer bioprocessing has largely been left unexplored both experimentally and industrially. Fortunately, the introduction of the innovative research tools is enabling research and development in many fields within the biotechnology and food technology spectra, specifically, indigenous fermented foods and beverages [1,7,12,14]. In summary, the possible food and beverage biotechnological advancements are endless, despite the opinions of many critics who believe that potential functional foods, such as *umqombothi*, should be put to rest [1,7,8,26]. Scientific enquiry in its comprehensiveness can prompt further studies focused on identifying important dynamics involved in food matrices [73]. Furthermore, better understanding of *umqombothi* and its interaction with man-made processes in a laboratory setting can be translated into industrial success [26,27]. Finally, producers and consumers can and should enjoy the abundance of *umqombothi* and other indigenous fermented products.

To achieve this, we advocate for necessary steps to ensure safety and the coupling of existing as well as emerging technologies for improved production efficiency, nutritional value and safety of *umqombothi*. As a result, optimisation approaches such as RSM, ANN and GA, as well as microbial analysis methods such as real-time PCR, metagenomics and 3D cell culturing, are useful technologies for advancing this beer type. For *umqombothi*, a two-step or three-step bioprocess optimisation approaches involving RSM, ANN and GA in combination with functional metagenomics will yield an easy-to-produce, nutritious, and safe end product.

Author Contributions: Conceptualisation, E.H., J.A.A. and O.A.A.; writing—original draft preparation, E.H.; writing—review and editing, E.H., J.A.A., W.D. and O.A.A.; supervision, J.A.A., W.D. and O.A.A.; funding acquisition, O.A.A. All authors have read and agreed to the published version of the manuscript.

Funding: The authors wish to acknowledge the financial support of the University of Johannesburg Global Excellence and Stature (GES) Masters' Fellowship, GES 4.0 Catalytic Initiative Grant and the NRF Thuthuka Grant (Grant number: 121826).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Konfo, C.T.R.; Chabi, N.W.; Dahouenon-Ahoussi, E.; Cakpo-Chichi, M.; Soumanou, M.M.; Sohounhloue, D.C.K. Improvement of african traditional sorghum beers quality and potential applications of plants extracts for their stabilization: A review. *J. Microbiol. Biotechnol. Food Sci.* **2015**, *5*, 190–196. [\[CrossRef\]](#)

2. Lues, J.; Ikalafeng, B.; Maharaso, M.; Shale, K. Brewing and Consumptions Practices of Indigenous Traditional Beer in A Typical South African Semi-Urban Area. *Indilinga Afr. J. Indig. Knowl. Syst.* **2009**, *8*, 163–170.
3. Sohrabvandi, S.; Mousavi, S.M.; Razavi, S.H.; Mortazavian, A.M. Application of Advanced Instrumental Techniques for Analysis of Physical and Physicochemical Properties of Beer: A Review. *Int. J. Food Prop.* **2010**, *13*, 744–759. [[CrossRef](#)]
4. Madella, M.; García-Granero, J.J.; Out, W.A.; Ryan, P.; Usai, D. Microbotanical Evidence of Domestic Cereals in Africa 7000 Years Ago. *PLoS ONE* **2014**, *9*, e110177. [[CrossRef](#)]
5. Wood-Sichra, U. *Cereal Crops*, 1st ed.; International Food Policy Research Institute (IFPRI): Washington, DC, USA, 2014; pp. 20–21.
6. Evera, E.; Abedin Abdallah, S.H.; Shuang, Z.; Sainan, W.; Yu, H. Shelf life and nutritional quality of sorghum beer: Potentials of phytogenic-based extracts. *J. Agric. Food. Tech.* **2019**, *9*, 1–14.
7. Adebo, O.A. African Sorghum-Based Fermented Foods: Past, Current and Future Prospects. *Nutrients* **2020**, *12*, 1111. [[CrossRef](#)] [[PubMed](#)]
8. Lyumugabe, F.; Gros, J.; Nzungize, J.; Bajyana, E.; Thonart, P. Characteristics of African traditional beers brewed with sorghum malt: A review. *Biotechnol. Agron. Soc. Environ.* **2012**, *16*, 509–530.
9. Gadaga, T.; Mutukumira, A.; Narvhus, J.; Feresu, S. A review of traditional fermented foods and beverages of Zimbabwe. *Int. J. Food Microbiol.* **1999**, *53*, 1–11. [[CrossRef](#)]
10. Adekoya, I.; Obadina, A.; Adaku, C.C.; De Boevre, M.; Okoth, S.; De Saeger, S.; Njobeh, P.B. Mycobiota and co-occurrence of mycotoxins in South African maize-based opaque beer. *Int. J. Food Microbiol.* **2018**, *270*, 22–30. [[CrossRef](#)] [[PubMed](#)]
11. Mboti, N. Song and genocide: Investigating the function of Yvonne Chaka Chaka’s ‘Umqombothi’ in Hotel Rwanda. *South-North Cult. Media Stud.* **2012**, *26*, 728–744. [[CrossRef](#)]
12. Ikalafeng, B.K. *Microbiota and Mycotoxins in Traditional Beer of The Greater Kimberley Area and Associated Brewing and Consumption Practices*; Central University of Technology: Bloemfontein, South Africa, 2008.
13. Choma, S.S.R.; Alberts, M.; Urdal, P. Effect of traditional beer consumption on the iron status of a rural South African population. *S. Afr. J. Clin. Nutr.* **2007**, *20*, 62–68. [[CrossRef](#)]
14. Katongole, J. *The Microbial Succession in Indigenous Fermented Maize Products*; University of Free State: Bloemfontein, South Africa, 2008.
15. 3 More Dead in EC After Drinking Home-Brewed Beer Mixed with Methylated Spirits. Available online: <https://www.news24.com/news24/southafrica/news/3-more-dead-in-ec-after-drinking-home-brewed-beer-mixed-with-methylated-spirits-20200524> (accessed on 19 August 2020).
16. Mawonike, R.; Chigunyeni, B.; Chipumuro, M. Process improvement of opaque beer (chibuku) based on multivariate cumulative sum control chart. *J. Inst. Brew.* **2017**, *124*, 16–22. [[CrossRef](#)]
17. Shackelton, S.E. *The Informal Marula Beer Traders of Bushbuckridge*; Rhodes University: Grahamstown, South Africa, 2003.
18. Umoja Adds Innovation to SA’s Umqombothi Heritage. Available online: <https://transformsa.co.za/2019/10/umoja-adds-innovation-to-sas-umqombothi-heritage/> (accessed on 17 August 2020).
19. Sorgho Looks to Modernise Umqombothi With Umoja Beer Powder. Available online: <https://retailbriefafrica.co.za/sorgho-looks-to-modernise-umqombothi-with-umoja-beer-powder/> (accessed on 17 August 2020).
20. Mandishona, E.; Moyo, V.; Gordeuk, V.; Khumalo, H.; Saungweme, T.; Gangaidzo, I.; Gomo, Z.; Rouault, T.; MacPhail, A. A traditional beverage prevents iron deficiency in African women of child bearing age. *Eur. J. Clin. Nutr.* **1999**, *53*, 722–725. [[CrossRef](#)] [[PubMed](#)]
21. Aloys, N.; Angeline, N. Traditional fermented foods and beverages in Burundi. *Food Res. Int.* **2009**, *42*, 588–594. [[CrossRef](#)]
22. Achi, O. The potential for upgrading traditional fermented foods through biotechnology. *Afr. J. Biotechnol.* **2005**, *4*, 375–380.
23. Lyumugabe, F.; Uyisenga, J.P.; Songa, E.B.; Thonart, P. Production of Traditional Sorghum Beer “Ikigage” Using *Saccharomyces cerevisiae*, *Lactobacillus fermentum* and *Issatchenkia orientalis* as Starter Cultures. *Food Nutr. Sci.* **2014**, *5*, 507–515. [[CrossRef](#)]
24. Moodley, S.S.; Dlamini, N.R.; Steenkamp, L.; Buys, E.M. Bacteria and yeast isolation and characterisation from a South African fermented beverage. *S. Afr. J. Sci.* **2019**, *115*, 1–6. [[CrossRef](#)]

25. Rogerson, C.M. African traditional beer: Changing organization and spaces of South Africa's sorghum beer industry. *Afr. Geogr. Rev.* **2019**, *38*, 253–267. [CrossRef]
26. Ronquest-Ross, L.-C.; Vink, N.; Sigge, G. Food consumption changes in South Africa since 1994. *S. Afr. J. Sci.* **2015**, *111*, 1–12. [CrossRef]
27. Nanadoum, M.; Pourquie, J. Sorghum Beer: Production, Nutritional Value and Impact upon Human Health. In *Beer in Health and Disease Prevention*; Elsevier: Amsterdam, The Netherlands, 2009; pp. 53–60.
28. The Oxford Companion to Beer Definition of Africa, Traditional Brewing In. Available online: <https://beerandbrewing.com/dictionary/izd8yFIQEc/> (accessed on 28 July 2020).
29. How to Make Umqombothi (Zulu Beer). Available online: <https://www.thesouthafrican.com/food/recipes/how-to-make-umqombothi/> (accessed on 28 July 2020).
30. United National Breweries—About Beer. Available online: <http://www.unbreweries.co.za/view/unb/en/page59.html> (accessed on 28 July 2020).
31. How Beer Is Made. Available online: <http://www.sab.co.za/post/beer-culture/how-beer-is-made> (accessed on 28 July 2020).
32. Ingredients to the Perfect Umqombothi. Available online: <https://www.jacarandafm.com/shows/the-complimentary-breakfast-with-rian-van-heerden/5-ingredients-perfect-umqombothi/> (accessed on 3 September 2020).
33. Lukinac, J.; Habschied, K.; Mastanjević, K.; Nakov, G.; Jukić, M. Computer Vision Method in Beer Quality Evaluation—A Review. *Beverages* **2019**, *5*, 38. [CrossRef]
34. Abdoul-latif, F.; Bassolé, I.; Dicko, M. Proximate composition of traditional local sorghum beer “dolo” manufactured in Ouagadougou. *Afr. J. Biotechnol.* **2013**, *12*, 1517–1522.
35. Wu, F.; Xu, X. Sprouted grains-based fermented products. *Sprouted Grains* **2019**, *7*, 143–173.
36. Kennedy, D.O. B Vitamins and the Brain: Mechanisms, Dose and Efficacy—A Review. *Nutrients* **2016**, *8*, 68. [CrossRef] [PubMed]
37. Yolmeh, M.; Jafari, S.M. Applications of Response Surface Methodology in the Food Industry Processes. *Food Bioprocess Technol.* **2017**, *10*, 413–433. [CrossRef]
38. Walsh, A.M.; Crispie, F.; Daari, K.; O'Sullivan, O.; Martin, J.C.; Arthur, C.T.; Claesson, M.J.; Scott, K.P.; Cotter, P.D. Strain-Level Metagenomic Analysis of the Fermented Dairy Beverage Nunu Highlights Potential Food Safety Risks. *Appl. Environ. Microbiol.* **2017**, *83*, 1111. [CrossRef]
39. Hou, Y.; Yin, Y.; Wu, G. Dietary essentiality of “nutritionally non-essential amino acids” for animals and humans. *Exp. Biol. Med.* **2015**, *240*, 997–1007. [CrossRef]
40. Hou, Y.; Wu, G. Nutritionally Essential Amino Acids. *Adv. Nutr.* **2018**, *9*, 849–851. [CrossRef]
41. Choi, B.-H.; Coloff, J.L. The Diverse Functions of Non-Essential Amino Acids in Cancer. *Cancers* **2019**, *11*, 675. [CrossRef]
42. Romano, C.; Corsetti, G.; Flati, V.; Pasini, E.; Picca, A.; Calvani, R.; Marzetti, E.; Dioguardi, F.S. Influence of Diets with Varying Essential/Nonessential Amino Acid Ratios on Mouse Lifespan. *Nutrients* **2019**, *11*, 1367. [CrossRef]
43. Mokoena, M.P.; Mutanda, T.; Olaniran, A.O. Perspectives on the probiotic potential of lactic acid bacteria from African traditional fermented foods and beverages. *Food Nutr. Res.* **2016**, *60*, 29630. [CrossRef]
44. Van Heerden, I. Nutrient content of sorghum beer strainings. *S. Afr. J. Anim. Sci.* **1987**, *17*, 171–175.
45. Van Heerden, I.V. The nutritive content of african beers brewed with maize grits or sorghum adjunct. *J. Inst. Brew.* **1989**, *95*, 17–20. [CrossRef]
46. Anderson, J.W.; Baird, P.; Davis, R., Jr.; Ferreri, S.; Knudtson, M.; Koraym, A.; Waters, V.; Williams, C.L. Health benefits of dietary fiber. *Nutr. Rev.* **2009**, *67*, 188–205. [CrossRef] [PubMed]
47. Kaczmarczyk, M.M.; Miller, M.J.; Freund, G.G. The health benefits of dietary fiber: Beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism* **2012**, *61*, 1058–1066. [CrossRef] [PubMed]
48. Ötles, S.; Ozgoz, S. Health effects of dietary fiber. *Acta Sci. Pol. Technol. Aliment.* **2014**, *13*, 191–202. [CrossRef] [PubMed]
49. Selhub, E.M.; Logan, A.C.; Bested, A.C. Fermented foods, microbiota, and mental health: Ancient practice meets nutritional psychiatry. *J. Physiol. Anthr.* **2014**, *33*, 2. [CrossRef] [PubMed]
50. Kim, B.; Hong, V.M.; Yang, J.; Hyun, H.; Im, J.J.; Hwang, J.; Yoon, S.; Kim, J.E. A Review of Fermented Foods with Beneficial Effects on Brain and Cognitive Function. *Prev. Nutr. Food Sci.* **2016**, *21*, 297–309. [CrossRef]

51. Şanlıer, N.; Gökçen, B.B.; Sezgin, A.C. Health benefits of fermented foods. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 506–527. [[CrossRef](#)]
52. Aslam, H.; Green, J.; Jacka, F.N.; Collier, F.; Berk, M.; Pasco, J.; Dawson, S.L. Fermented foods, the gut and mental health: A mechanistic overview with implications for depression and anxiety. *Nutr. Neurosci.* **2020**, *23*, 659–671. [[CrossRef](#)]
53. Sivamaruthi, B.S.; Kesika, P.; Chaiyasut, C. Impact of Fermented Foods on Human Cognitive Function—A Review of Outcome of Clinical Trials. *Sci. Pharm.* **2018**, *86*, 22. [[CrossRef](#)]
54. Van Der Walt, J.P. Kaffir corn malting and brewing studies. II.—Studies on the microbiology of Kaffir beer. *J. Sci. Food Agric.* **1956**, *7*, 105–113. [[CrossRef](#)]
55. Van Der Walt, J. Better Kaffir beer. *S. Afr. J. Sci.* **1954**, *50*, 221.
56. Capozzi, V.; Fragasso, M.; Romaniello, R.; Berbegal, C.; Russo, P.; Spano, G. Spontaneous Food Fermentations and Potential Risks for Human Health. *Fermentation* **2017**, *3*, 49. [[CrossRef](#)]
57. Berbegal, C.; Khomenko, I.; Russo, P.; Spano, G.; Fragasso, M.; Biasioli, F.; Capozzi, V. PTR-ToF-MS for the Online Monitoring of Alcoholic Fermentation in Wine: Assessment of VOCs Variability Associated with Different Combinations of *Saccharomyces*/Non-*Saccharomyces* as a Case-Study. *Fermentation* **2020**, *6*, 55. [[CrossRef](#)]
58. Capozzi, V.; Fragasso, M.; Russo, P. Microbiological Safety and the Management of Microbial Resources in Artisanal Foods and Beverages: The Need for a Transdisciplinary Assessment to Conciliate Actual Trends and Risks Avoidance. *Microorganisms* **2020**, *8*, 306. [[CrossRef](#)]
59. Nyanzi, R.; Jooste, P. Cereal-Based Functional Foods. *Probiotics* **2012**, 161–197. [[CrossRef](#)]
60. Shephard, G.S.; Van Der Westhuizen, L.; Gatyeni, P.M.; Somdya, N.I.M.; Burger, H.-M.; Marasas, W.F.O. Fumonisin Mycotoxins in Traditional Xhosa Maize Beer in South Africa. *J. Agric. Food Chem.* **2005**, *53*, 9634–9637. [[CrossRef](#)]
61. Van Hijum, S.A.; Vaughan, E.E.; Vogel, R.F. Application of state-of-art sequencing technologies to indigenous food fermentations. *Curr. Opin. Biotechnol.* **2013**, *24*, 178–186. [[CrossRef](#)]
62. Young, L.M.; Pipingas, A.; White, D.; Gauci, S.; Scholey, A.B. A Systematic Review and Meta-Analysis of B Vitamin Supplementation on Depressive Symptoms, Anxiety, and Stress: Effects on Healthy and ‘At-Risk’ Individuals. *Nutrients* **2019**, *11*, 2232. [[CrossRef](#)]
63. Behera, S.S.; Ray, R.C.; Zdolec, N. *Lactobacillus plantarum* with Functional Properties: An Approach to Increase Safety and Shelf-Life of Fermented Foods. *BioMed Res. Int.* **2018**, *2018*, 9361614. [[CrossRef](#)]
64. Fang, F.; Xu, J.; Li, Q.; Xia, X.; Du, G. Characterization of a *Lactobacillus brevis* strain with potential oral probiotic properties. *BMC Microbiol.* **2018**, *18*, 1–9. [[CrossRef](#)] [[PubMed](#)]
65. Naghmouchi, K.; Belguesmia, Y.; Bendali, F.; Spano, G.; Seal, B.S.; Drider, D. *Lactobacillus fermentum*: A bacterial species with potential for food preservation and biomedical applications. *Crit. Rev. Food Sci. Nutr.* **2019**, *1*, 1–13. [[CrossRef](#)] [[PubMed](#)]
66. Ercolini, D. High-Throughput Sequencing and Metagenomics: Moving Forward in the Culture-Independent Analysis of Food Microbial Ecology. *Appl. Environ. Microbiol.* **2013**, *79*, 3148–3155. [[CrossRef](#)] [[PubMed](#)]
67. Staley, C.; Sadowsky, M.J. Application of metagenomics to assess microbial communities in water and other environmental matrices. *J. Mar. Biol. Assoc. UK* **2015**, *96*, 121–129. [[CrossRef](#)]
68. Berbegal, C.; Borruso, L.; Fragasso, M.; Tufariello, M.; Russo, P.; Tognetti, R.; Spano, G.; Capozzi, V. A Metagenomic-Based Approach for the Characterization of Bacterial Diversity Associated with Spontaneous Malolactic Fermentations in Wine. *Int. J. Mol. Sci.* **2019**, *20*, 3980. [[CrossRef](#)]
69. Russo, P.; Fiocco, D.; Albenzio, M.; Spano, G.; Capozzi, V. Microbial Populations of Fresh and Cold Stored Donkey Milk by High-Throughput Sequencing Provide Indication for A Correct Management of This High-Value Product. *Appl. Sci.* **2020**, *10*, 2314. [[CrossRef](#)]
70. Makinde, O.M.; Ayeeni, K.I.; Sulyok, M.; Krska, R.; Adeleke, R.A.; Ezekiel, C.N. Microbiological safety of ready-to-eat foods in low-and middle-income countries: A comprehensive 10-year (2009 to 2018) review. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 703–732. [[CrossRef](#)]
71. Leonard, S.R.; Mammel, M.K.; Lacher, D.W.; Elkins, C.A. Application of Metagenomic Sequencing to Food Safety: Detection of Shiga Toxin-Producing *Escherichia coli* on Fresh Bagged Spinach. *Appl. Environ. Microbiol.* **2015**, *81*, 8183–8191. [[CrossRef](#)]
72. Takahashi, M.B.; De Oliveira, H.C.; Núñez, E.G.F.; Rocha, J.C. Brewing process optimization by artificial neural network and evolutionary algorithm approach. *J. Food Process. Eng.* **2019**, *42*, e13103. [[CrossRef](#)]

73. Chibundu, N.E.; Kolawole, I.A.; Jane, M.M.; Yinka, M.S.; Ihuoma, E.C.; Oluwawapelumi, A.O.; Wilfred, A.A.; Michael, S.; Gordon, S.S.; Rudolf, K. Traditionally Processed Beverages in Africa: A Review of the Mycotoxin Occurrence Patterns and Exposure Assessment. *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 334–351.
74. Burger, H.-M.; Lombard, M.; Shephard, G.S.; Rheeder, J.; Van Der Westhuizen, L.; Gelderblom, W. Dietary fumonisin exposure in a rural population of South Africa. *Food Chem. Toxicol.* **2010**, *48*, 2103–2108. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Matsheka, M.; Magwamba, C.; Mpuchane, S.; Gashe, B. Biogenic amine producing bacteria associated with three different commercially fermented beverages in Botswana. *Afr. J. Microbiol. Res.* **2013**, *7*, 342–350. [\[CrossRef\]](#)
76. Magwamba, C.; Matsheka, M.I.; Mpuchane, S.; Gashe, B.A. Detection and Quantification of Biogenic Amines in Fermented Food Products Sold in Botswana. *J. Food Prot.* **2010**, *73*, 1703–1708. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Lasekan, O. Biogenic amines in traditional alcoholic beverages produced in Nigeria. *Food Chem.* **2000**, *69*, 267–271. [\[CrossRef\]](#)
78. Isaacson, C. The change of the staple diet of black South Africans from sorghum to maize (corn) is the cause of the epidemic of squamous carcinoma of the oesophagus. *Med. Hypotheses* **2005**, *64*, 658–660. [\[CrossRef\]](#)
79. Isaacson, C.; Mothobi, P.; Hale, M.; Tomar, L.K.; Tyagi, C.; Kumar, P.; Choonara, Y.E.; Altini, M.; Pillay, V. Carcinogenic nitrosamines in traditional beer as the cause of oesophageal squamous cell carcinoma in black South Africans. *S. Afr. Med J.* **2015**, *105*, 656. [\[CrossRef\]](#)
80. Sate Wants A Say in How to Make Beer. Available online: <https://www.news24.com/news24/southafrica/news/state-wants-a-say-in-how-to-make-beer-20170722> (accessed on 8 September 2020).
81. Kana, E.B.G.; Oloke, J.K.; Lateef, A.; Oyeibanji, A. Comparative evaluation of artificial neural network coupled genetic algorithm and response surface methodology for modeling and optimization of citric acid production by *Aspergillus niger* MCBN297. *Chem. Eng.* **2012**, *27*, 397–402.
82. Basheer, I.; Hajmeer, M. Artificial neural networks: Fundamentals, computing, design, and application. *J. Microbiol. Methods* **2000**, *43*, 3–31. [\[CrossRef\]](#)
83. Sewsynker-Sukai, Y.; Faloye, F.; Kana, E.B.G. Artificial neural networks: An efficient tool for modelling and optimization of biofuel production (a mini review). *Biotechnol. Biotechnol. Equip.* **2017**, *31*, 221–235. [\[CrossRef\]](#)
84. Madamba, P. The Response Surface Methodology: An Application to Optimize Dehydration Operations of Selected Agricultural Crops. *Lebensm. Wiss. Technol.* **2002**, *37*, 584–590. [\[CrossRef\]](#)
85. Nwabueze, T.U. Review article: Basic steps in adapting response surface methodology as mathematical modelling for bioprocess optimisation in the food systems. *Int. J. Food Sci. Technol.* **2010**, *45*, 1768–1776. [\[CrossRef\]](#)
86. Cioffi, R.; Travaglioni, M.; Piscitelli, G.; Petrillo, A.; De Felice, F. Artificial Intelligence and Machine Learning Applications in Smart Production: Progress, Trends, and Directions. *Sustainability* **2020**, *12*, 492. [\[CrossRef\]](#)
87. Simeone, O. A Very Brief Introduction to Machine Learning With Applications to Communication Systems. *IEEE Trans. Cogn. Commun. Netw.* **2018**, *4*, 648–664. [\[CrossRef\]](#)
88. Fatiha, B.; Sameh, B.; Youcef, S.; Zeineddine, D.; Nacer, R. Comparison of artificial neural network (ann) and response surface methodology (rsm) in optimization of the immobilization conditions for lipase from *Candida rugosa* ON AMBERJET®4200-CL. *Prep. Biochem. Biotechnol.* **2013**, *43*, 33–47. [\[CrossRef\]](#)
89. Reyed, R. Computational Biotechnology: An Approach in Silico Based Modeling Bioprocess. *Int. J. Res. Stud. Microbiol. Biotechnol.* **2019**, *5*, 1–5.
90. Ou, Y.; Wilson, R.; Weber, S. Methods of Measuring Enzyme Activity Ex Vivo and In Vivo. *Annu. Rev. Anal. Chem.* **2018**, *11*, 509–533. [\[CrossRef\]](#)
91. Kim, H. AI, big data, and robots for the evolution of biotechnology. *Genom. Inform.* **2019**, *17*, e44. [\[CrossRef\]](#)
92. Oliveira, A.L. Biotechnology, Big Data and Artificial Intelligence. *Biotechnol. J.* **2019**, *14*, e1800613. [\[CrossRef\]](#)
93. Salam, O.; Okwu, M. Comparison of Response Surface Methodology (RSM) and Artificial Neural Network (ANN) in modelling of waste coconut oil ethyl esters production. *Energy Sources Part A Recover. Util. Environ. Eff.* **2018**, *41*, 1049–1061. [\[CrossRef\]](#)
94. Dey, A. Machine learning algorithms: A review. *Int. J. Comput. Sci. Inf. Technol.* **2016**, *7*, 1174–1179.
95. Littmann, M.; Selig, K.; Cohen-Lavi, L.; Frank, Y.; Hönigschmid, P.; Kataka, E.; Mösch, A.; Qian, K.; Ron, A.; Schmid, S.; et al. Validity of machine learning in biology and medicine increased through collaborations across fields of expertise. *Nat. Mach. Intell.* **2020**, *2*, 18–24. [\[CrossRef\]](#)

96. Harsh, K.; Bharath, N.; Siddesh, C.; Kuldeep, S. An Introduction to Artificial Neural Network. *Int. J. Adv. Res. Innov. Ideas Educ.* **2016**, *1*, 27–30.
97. Mewa-Ngongang, M.; Du Plessis, H.W.; Ntwampe, S.; Chidi, B.S.; Hutchinson, U.F.; Mekuto, L.; Jolly, N.P. Grape Pomace Extracts as Fermentation Medium for the Production of Potential Biopreservation Compounds. *Foods* **2019**, *8*, 51. [[CrossRef](#)] [[PubMed](#)]
98. Mewa-Ngongang, M.; Du Plessis, H.W.; Hlangwani, E.; Ntwampe, S.; Chidi, B.S.; Hutchinson, U.F.; Jolly, N.P. Activity Interactions of Crude Biopreservatives against Spoilage Yeast Consortia. *Fermentation* **2019**, *5*, 53. [[CrossRef](#)]
99. Mandal, S.; Panda, A.; Bisht, S.; Kumar, N. Microbial Ecology in the Era of Next Generation Sequencing. *J. Next Gener. Seq. Appl.* **2015**, *1*, 1058–1066. [[CrossRef](#)]
100. Oulas, A.; Pavloudi, C.; Polymenakou, P.; Pavlopoulos, G.A.; Papanikolaou, N.; Kotoulas, G.; Arvanitidis, C.; Iliopoulos, L. Metagenomics: Tools and Insights for Analyzing Next-Generation Sequencing Data Derived from Biodiversity Studies. *Bioinform. Biol. Insights* **2015**, *9*, 75–88. [[CrossRef](#)] [[PubMed](#)]
101. Adebo, O.A.; Oyeyinka, S.A.; Adebisi, J.A.; Feng, X.; Wilkin, J.D.; Kewuyemi, Y.O.; Abrahams, A.M.; Tugizimana, F. Application of gas chromatography–mass spectrometry (GC-MS)-based metabolomics for the study of fermented cereal and legume foods: A review. *Int. J. Food Sci. Technol.* **2020**. [[CrossRef](#)]

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).