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Nutritional Compositions of Optimally Processed *Umqombothi* (a South African Indigenous Beer)

Edwin Hlangwani, Janet Adeyinka Adebiyi * and Oluwafemi Ayodeji Adebo *

Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, Doornfontein Campus, P.O. Box 17011, Doornfontein 2028, South Africa; eddnathan.muk@gmail.com
* Correspondence: janetaadex@gmail.com (J.A.A.); oadebo@uj.ac.za (O.A.A.)

Abstract: *Umqombothi* (a South African indigenous beer) is an important dietary beverage for many undernourished, low-income consumers in rural, semi-urban and urban areas. *Umqombothi* was brewed using optimal conditions earlier obtained and compared to the customary beer brew (CB) and mixed raw ingredients (RI). The products were evaluated for proximate compositions, minerals, amino acids, B-group vitamins, and sugar compounds. The optimised beer brew (OPB) was relatively higher in energy (165 kcal), crude protein (8.6%), and ash content (1.0%). The CB had the highest concentration of sodium (299.8 mg/kg), magnesium (1170.5 mg/kg), potassium (2993.8 mg/kg), and phosphorus (2100.7 mg/kg). Glutamic acid was the highest detected amino acid, with concentrations of 1.5 g/100 g, 1.5 g/100 g, and 1.6 g/100 g in the RI, CB, and OPB, respectively. The OPB contained a higher concentration of the two forms of vitamin B_3, nicotinamide (0.2 µg/g) and nicotinic acid (0.7 µg/g) in comparison to the CB. The concentration of the antioxidant, mannitol, was 0.4 mg/g, 0.2 mg/g, and 2.0 mg/g in the RI, CB, and OPB respectively. Overall, OPB displayed a desirable nutritional profile compared to the CB.

Keywords: beverage; compositions; diet; health; nutrition; *umqombothi*

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1. Introduction

Traditional beers have both significant socio-cultural, economic, and nutritional value in Africa [1,2]. The diversity of the nutritional profile of a particular beer product is a result of the choice of raw materials, fermentation process, and processing techniques employed [3]. Traditionally processed African beers lack consistent nutritional compositions due to variability in the preparation conditions which are dependent on the knowledge, skills, and expertise of the brewers [4,5]. Nonetheless, local cultures are rich with the consumption of sorghum, millet, and wheat-based fermented beverages prepared on a household level or by small-scale industries [6].

African opaque beers such as *umqombothi* common to South Africa have been reported to contain significant amounts of B-group vitamins, amino acids, dietary fibre, minerals, protein, and carbohydrates [7–9]. As a result, *umqombothi* is enjoyed by people of diverse social classes, age groups, and sexual orientations [3,10,11]. More importantly, *umqombothi*'s high caloric density is a convenient, affordable, and readily consumable source of energy in communities where poverty and malnutrition are common [3,5]. This may also explain why *umqombothi* is often consumed after cooperative work, festivals, and tribal meetings [12].

Unfortunately, data describing the nutritional composition of local beers is limited [4,8]. As a result, misinformation on nutritional information is rampant across African communities, especially in South Africa [13,14]. In light of this, a large opportunity exists to inform consumers about the nutritional composition of optimally processed *umqombothi* [15]. Consumers find scientific studies to be the most credible sources of nutritional information about alcoholic beverages and this study thus investigated the nutritional compositions of optimally processed *umqombothi* in comparison to the nutritional compositions of mixed raw ingredients and a customary beer brew.

2. Materials and Methods

2.1. Traditional Beer (Umqombothi) Brewing Process

The brewing process followed a method described by [16] whereby 500 g of King Korn malted sorghum (*Mtombo-Mmela*) (Tiger Brands, Johannesburg, South Africa) was mixed with 1000 g of White Star maize meal (Pioneer Foods, Paarl, South Africa) in a sterile 10 L bucket filled with 7 L sterile tap water. The mixture was gently stirred, covered, and incubated at 25 °C for 24 h to sour. The optimised beer brew (OPB) was prepared by cooking the mixed ingredients for 1.1 h at 95 °C and allowed to cool to 25 °C. To the cooled porridge, 500 g of King Korn malted sorghum (*Mtombo-Mmela*) (Tiger Brands, Johannesburg, South Africa) was added and homogenised with gentle stirring. The mixture was then fermented at 29.3 °C for 25.9 h to obtain the OPB. On the other hand, the customary brew (CB) was prepared by cooking the mixed ingredients for 30 min at 95 °C and allowed to cool to 25 °C. To the cooled porridge, 500 g of King Korn malted sorghum (*Mtombo-Mmela*) (Tiger Brands, Johannesburg, South Africa) was added and homogenised with gentle stirring and subsequently fermented at 25 °C for 24 h. The choice of fermentation time and temperature conditions for CB was guided by available studies on *umqombothi* processing [2,5,8], while that of OPB was based on an earlier study where fermentation conditions for *umqombothi* processing were optimized [16]. The mixed raw ingredients (RI) were prepared by mixing 500 g of King Korn malted sorghum (*Mtombo-Mmela*) (Tiger Brands, Johannesburg, South Africa), 1000 g of White Star maize meal (Pioneer Foods, Paarl, South Africa), and 7 L tap water.

2.2. Sample Preparation

To prepare the RI samples, 35 mL of the unfiltered mixture of 500 g of King Korn malted sorghum (*Mtombo-Mmela*) (Tiger Brands, Johannesburg, South Africa), 1000 g of White Star maize meal (Pioneer Foods, Paarl, South Africa), and 7 L water was transferred into sterile conical tubes and freeze-dried (LyoQuest laboratory freeze dryer, Telstar, Terrassa, Spain) for 72 h. Thereafter, the dry samples were milled and weighed into marked zip lock bags with respect to the requirements of prospective analyses (Sections 2.3–2.7). The OPB and CB samples were each prepared following the brewing conditions previously described (Section 2.1). Thereafter, separate sterile conical tubes were each used to collect 35 mL of unfiltered CB and OPB samples immediately after the completion of their respective fermentation processes (i.e., OPB fermentation conditions = 25.9 h at 29.3 °C; CB fermentation conditions = 24 h at 25 °C) and freeze-dried (LyoQuest laboratory freeze dryer, Telstar, Terrassa, Spain) for 72 h. The dry OPB and CB samples were then milled and weighed into marked zip lock bags as per the requirements of prospective analyses (Sections 2.3–2.7). Finally, as dictated by the type of analysis, equal amounts of RI, CB, and OPB samples were analysed on a dry matter (DM) basis.

2.3. Proximate Compositions and Total Energy Determination

Crude protein, crude fibre, crude fat, moisture, and ash were respectively determined on a DM basis using [17] methods 990.03, 978.10, 920.39 (A), 934.01, and 923.03. Thereafter, the carbohydrate content was determined by difference as described by [18]. Lastly, Atwater factors were used to calculate the total energy whereby the energy value (kcal) = (% protein × 4 + % carbohydrate × 4 + % fat × 9.0) [19].

2.4. Mineral Compositions Determination Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

To prepare each sample type (i.e., RI, CB, and OPB) for microwave digestion, 10 mL nitric acid (16 M) (Sigma-Aldrich, St. Louise, MO, USA) and 0.5 g of the corresponding freeze-dried (LyoQuest laboratory freeze dryer, Telstar, Terrassa, Spain) sample type was carefully mixed in Teflon tubes (MARSXpress—High Throughput Vessels). The microwave digester (CEM One TouchTM Technology, CEM Technologies, North Carolina, USA), programmed to run at a temperature of 25–170 °C for 10 min and an additional 10 min

at 170–240 °C at 1 kW, followed by ventilation at room temperature (25 °C) for 20 min, was used to digest all the prepared samples. The resulting solutions were allowed to cool and made up to mark with ultrapure water (Millipore Sigma, Bedford, USA) in a 50 mL volumetric flask. Inductively coupled plasma mass spectrometry (ICP-MS) standard solutions: calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), phosphorus (P), sodium (Na), sulfur (S) and zinc (Zn) were used for preparing working standard solutions and ICP-MS analysis stock solutions. Internal standards were Germanium (Ge), Rhodium (Rh), and Scandium (Sc). The prepared samples were analysed in triplicate on an ICP-MS equipment (Spectro ARCOS, Spectro Analytical Instruments, Kleve, Germany). The conditions and parameters of the instrument were as follows: auxiliary gas flow—0.5 L/min; gas (at 600 kPa)—argon; nebulizer flow—0.5 L/min; nebulizer type—concentric; PMT volts—600 V; RF power—1200; rinse time—5 min; sample flow—0.9 mL/min. Ca (317.93), Cr (267.72), Cu (327.40), Fe (259.94), K (766.49), Mg (285.21), Mn (257.61), Mo (202.10), Na (589.59), P (178.29), S (182.03) and Zn (206.20) were wavelengths (nm) used for the analysis.

2.5. Determination of Amino Acid Compositions

The method described by [20] was used for amino acid determination. The analysis was performed using high-performance liquid chromatography (HPLC) at the Agricultural Research Council (ARC)-Irene Analytical Research Laboratory (Pretoria, South Africa). The samples were prepared by hydrolysing an internal standard of α-amino-β-guanidino propionic acid with 700 mg of each sample using equal volumes of hydrochloric acid (6 N) solution. Thereafter, each hydrolysate was transferred to clean Eppendorf tubes and centrifuged (Eppendorf, Hamburg, Germany) at 2000× *g* for 10 min. Using a 0.45 µm filter, the supernatant was removed. The protein hydrolysate was dried under nitrogen gas and derivatized using fluorenylmethyloxycarbonyl chloride (FMOC) reagent (Sigma-Aldrich, St. Louise, MO, USA) and borate buffer. The resulting derivatized mixture extracted with pentane was analyzed using HPLC with fluorescence detectors (Schoeffel FS 970, Perkin-Elmer LS-4, and Shimadzu RF-530) at an excitation wavelength of 260 nm and an emission wavelength of 313 nm. The mobile phase used as the eluent was a mixture of acetic acid, methanol, and acetonitrile (50:40:10, *v*/*v*/*v*) and was varied linearly to acetonitrile:acetic acid (50:50, *v*/*v*) over 90 min. The oven temperature was kept at 40 °C while the gradient flow was started at 3 min at 1.3 mL/min flow rate, which was later increased to 2 mL/min for 0.5 min at the end of the gradient. To generate calibration curves and determine the concentration of the amino acids in the prepared samples, amino acid standards (Sigma-Aldrich, St. Louise, MO, USA) were used.

2.6. Determination of Sugar Compounds

Samples were prepared and subsequently injected into a gas chromatograph (TRACE1300, Thermo Scientific, Waltham, MA, USA) coupled to a flame ionization detector (FID). To 20 mg of each sample type (i.e., RI, CB, and OPB), 1 mL of methanol (70%) was added, mixed, and extracted in an oven set at a temperature of 60 °C for 3–4 h. Thereafter, 250 µL of each sample type was completely dried under a gentle stream of nitrogen gas. Exactly 0.1 mL of 2% methoxyamine in pyridine (Sigma-Aldrich, St. Louise, MO, USA) was used to derivatize each sample at 40 °C for 2 h. Thereafter, 0.05 mL N,O-bis(trimethylsilyl)trifluoroacetamide) (Sigma-Aldrich, St. Louise, MO, USA) was added and re-derivatized at 60 °C for 30 min, vortexed, transferred to an insert, and injected into a GC-MS instrument. The GC-FID system was connected to a CTC Analytics PAL autosampler (Agilent, Santa Clara, CA, USA). Separation of sugars was performed on a Zebron Semi-volatile (30 m, 0.25 mm ID, 0.25 µm film thickness) 7HG-G027-11-GGA capillary column (Phenomenex, California, USA). Helium was used as the carrier gas at a flow rate of 1 mL/min and injector temperature was maintained at 240 °C. One µL of each sample type was injected in a 10:1 split ratio. The oven temperature was programmed

as follows: 80 °C for 1 min and ramped up to 300 °C at a rate of 7 °C/min for 2 min. The temperature of the FID was maintained at 300 °C.

2.7. Determination of B-Group Vitamins

To extract the vitamins, 1 g of each sample type (i.e., RI, CB, and OPB) was weighed into 50 mL conical tubes. To the dried powder, 30 mL of water:acetonitrile (95:5 v/v) with acetic acid 1% (v/v) was added. The mixture was vortexed and incubated in a water bath (Labcon, Chamdor, South Africa) set at 50 °C for 10 min. Thereafter, the samples were cooled and centrifuged (Eppendorf, Hamburg, Germany) at 2.333.3× *g* for 10 min. Finally, the supernatant was filtered through a 0.22 µm syringe filter. The vitamins were then quantified using a high-performance liquid chromatography-mass spectrometer (HPLC-MS) (Shimadzu Corporation, Tokyo, Japan), with an LC-30AD Nexera chromatograph connected to an autosampler (SIL-30 AC) and a column oven (CTO-20 AC), maintained at a temperature of 40 °C. The chromatographic separation of B-group vitamins was achieved on a Raptor ARC-18 column (2.7 µm, 2.1 mm × 100 mm) (Restek Corporation, Bellefonte, PA, USA). The injection volume of the sample was 2 µL, and the mobile phases were: 0.1% formic acid (FA) in ultrapure water (solvent A), and methanol with 0.1% formic acid (solvent B). The LC gradient program began with 99% of solvent A for 0.5 min, reduced to 60% of solvent A in 2 min, maintained at this condition for 1 min, ramped up to 95% of solvent A and taken back to the initial condition of 99% in 1 min. This condition was then maintained for 1 min to re-equilibrate the column for the next run. The mobile phase was delivered at a constant flow rate of 0.3 mL/min, with a total run time of 6 min. The LC was connected to a Shimadzu triple-quadrupole MS model 8030 (Shimadzu Corporation, Kyoto, Japan) detector with an electron spray ionization source where B-group vitamins were detected and quantified in positive ionization mode (ESI+). The MS method consisted of a multiple reaction monitoring (MRM) method operated at optimised MS conditions for the analytes. The desolvation line (DL) temperature was 250 °C, the heat block temperature was 400 °C, the interface nebulizing gas flow rate was 3 L/min, and drying gas flow rate was 15 L/min. The Shimadzu Lab Solutions software was used for data processing and visualization.

2.8. Statistical Analysis

All experiments were conducted in triplicate and expressed as mean ± standard deviation (S.D). Analysis of variance (ANOVA) was employed to determine the significance of the data using SPSS statistics 27.0 software (IBM, New York, NY, USA). Means were separated using Duncan's multiple range test (DMRT) and significant differences were accepted at $p < 0.05$.

3. Results

3.1. Proximate Compositions

The proximate composition on a DM basis of the two types of processed beer samples, together with mixed raw ingredients are shown in Table 1. As expected, a higher ash content was observed in the optimised beer brew (OPB) (1.0%) and customary beer brew (CB) (0.9%) compared to the mixed raw ingredients (RI) (0.7%). Fermentation conditions were ideal for rapid yeast biomass thereby increasing mineral and dry matter content which led to a higher ash content [21,22]. The ash content of 4.1% in *amgba* has been reported [3].

The crude protein was 8.6%, 8.1%, and 7.5% in the OPB, CB, and RI respectively (Table 1). The protein content in the raw material often ranged between 6 and 18%, depending on the type of grain (sorghum, maize, or millet) used [23]. A study by [24], found the protein content in *umqombothi* to be 0.5%. The higher protein values in this study could be due to the use of malted sorghum or genetically modified high-protein maize [25,26].

Table 1. Proximate compositions of *umqombothi*.

Parameters (%)	RI	CB	OPB
Ash	$0.7^{a} \pm 0.1$	$0.9^{b} \pm 0.0$	$1.0^{c} \pm 0.1$
Carbohydrate	$33.1^{c} \pm 1.7$	$30.7^{a} \pm 2.3$	$32.5^{b} \pm 0.2$
Crude fat	$0.2^{b} \pm 0.0$	$0.1^{a} \pm 0.1$	$0.1^{a} \pm 0.0$
Crude fibre	$50.7^{a} \pm 1.4$	$56.8^{c} \pm 2.4$	$54.6^{b} \pm 0.3$
Crude protein	$7.5^{a} \pm 0.1$	$8.1^{b} \pm 0.1$	$8.6^{c} \pm 0.4$
Moisture	$7.7^{c} \pm 0.3$	$3.4^{b} \pm 0.3$	$3.2^{a} \pm 0.1$
Energy value (kcal/100 g)	$164.5^{b} \pm 6.5$	$156.2^{a} \pm 9.6$	$165.0^{c} \pm 0.7$

RI—mixed raw ingredients; CB—customary beer brew; OPB—optimised beer brew. Mean values with different superscripts within the same row are significantly different at ($p < 0.05$).

The fibre content accounted for half the macromolecules for all the samples. The CB had the highest fibre content (56.8%), followed by the OPB (54.6%). The residual sorghum grains in *umqombothi* consist of solids made of dextrins, dietary fibres, and starch residues that give the beer its high fibre content [5,8,27]. The dietary fibre may impart the beer with antimicrobial properties, possibly inducing favourable bowel movements [28,29]. As a result, moderate and regular consumption of fibre-rich *umqombothi* may be beneficial in reducing the development of type-2 diabetes, gastroesophageal reflux disease, and duodenal ulcers [29–31].

Crude fat was the lowest macronutrient for all the samples, with the lowest concentrations observed in the OPB, CB, and mixed RI, respectively (Table 1). In beer, fat content is negligibly present in the form of fatty acids, thus having a minimal contribution to the energy value of the beverage [32–34]. Since fat is considered a trace constituent in beer, these findings were in line with other studies [3,33,35–37]. A fat content of 0% in *umqombothi* was also reported by [4].

The mixed RI showed the highest concentration of carbohydrates. The CB and OPB underwent fermentation and thus displayed a relatively lower carbohydrate content. In general, the lower the carbohydrate content, the higher the degree of fermentation of the beer [38,39]. For all the samples, the carbohydrate content in 100 g was within the range of 3.6–4% found by other authors [3,35,36]. A higher carbohydrate content in the RI was not surprising considering that the sorghum grain is made of about 75% starch [40]. Carbohydrates contribute significantly to the caloric density of *umqombothi*, which was highest in the OPB (165 kcal/100 g) [5,7,8]. This study was consistent with that of Van Heerden. [28], in that the beer was not filtered, hence the higher caloric value.

Lyumugabe et al. [3] observed a general calorie count for African traditional beer to be about 37 kcal/100 mL, while Abdoul-latif et al. [4] observed an energy value of 31.1 kcal/100 mL for *umqombothi* specifically. Other studies reported a calorie range of 31.1–466.5 kcal/100 mL [4,23,28]. The caloric density of *umqombothi* makes this beer an integral part of the diet for millions of poverty-stricken communities in Africa [11,41]. In many instances, the beer has been used as an energy drink before and after community work, cooperative work, or meetings of mutual associations [42,43].

3.2. Mineral Composition

All the samples were relatively high in essential macrominerals such as sodium, magnesium, phosphorus, sulfur, potassium, and calcium (Table 2). The CB samples, followed by the OPB samples displayed significantly higher mineral content than the RI. Since both the CB and OPB were fermented samples, antinutritional factors that may have limited mineral availability and detection by forming insoluble complexes within the food matrices were possibly degraded [9,16,40].

Table 2. Mineral compositions of *umqombothi* produced under optimum conditions.

Mineral (mg/kg)	RI	CB	OPB
As	ND	ND	ND
Ca	139.3 ^{a} ± 0.2	299.8 ^{c} ± 0.2	222.0 ^{b} ± 0.2
Co	ND	0.1 ^{a} ± 0.0	ND
Cr	0.2 ^{b} ± 0.0	0.2 ^{b} ± 0.0	0.1 ^{a} ± 0.0
Cu	1.6 ^{a} ± 0.0	2.8 ^{c} ± 0.0	2.5 ^{b} ± 0.0
Fe	31.6 ^{a} ± 0.0	44.1 ^{c} ± 0.0	36.0 ^{b} ± 0.0
K	1864.3 ^{a} ± 0.8	2993.8 ^{c} ± 1.4	2584.6 ^{b} ± 1.0
Mg	684.1 ^{a} ± 0.1	1170.5 ^{c} ± 0.1	1059.9 ^{b} ± 0.1
Mo	0.1 ^{a} ± 0.0	0.2 ^{b} ± 0.0	0.2 ^{b} ± 0.0
Mn	7.1 ^{a} ± 0.0	12.4 ^{c} ± 0.0	11.1 ^{b} ± 0.0
Na	22.4 ^{a} ± 0.0	68.3 ^{c} ± 0.0	56.2 ^{b} ± 0.0
Ni	0.5 ^{a} ± 0.0	0.8 ^{c} ± 0.0	0.6 ^{b} ± 0.0
P	1264.2 ^{a} ± 0.3	2100.7 ^{c} ± 0.6	1860.8 ^{b} ± 0.5
Pb	ND	ND	ND
S	5728.6 ^{c} ± 39.1	5047.7 ^{a} ± 3.8	5702.0 ^{b} ± 40.0
Se	ND	0.2 ^{a} ± 0.0	ND
Zn	21.7 ^{b} ± 0.0	22.1 ^{c} ± 0.0	19.3 ^{a} ± 0.0

As—arsenic; Ca—calcium; CB—customary beer brew; Co—cobalt; Cr—chromium; Cu—copper; Fe—iron; K—potassium; Mg—magnesium; Mn—manganese; Mo—molybdenum; Na—sodium; ND—not detected; Ni—nickel; OPB—optimised beer brew; P—phosphorus; Pb—lead; RI—mixed raw ingredients; S—sulfur; Se—selenium; Zn—zinc. Mean values with different superscripts within the same row are significantly different at ($p < 0.05$).

Potentially toxic elements, chromium, cobalt, nickel, arsenic, and lead were detected in significantly low levels. Nickel had the highest detection for potentially toxic elements (Table 2). Nonetheless, for each heavy metal, the detection was below the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [44,45]. Present in small, diet-common quantities, trace elements can be of nutritional benefit [46]. At Dikgale, Limpopo Province, South Africa, *umqombothi* consumption was shown to prevent iron deficiency in people at risk of the deficiency [42].

3.3. Amino Acid (AA) Compositions

Glutamic acid was the most abundant AA, with a high concentration observed in the OPB (Table 3). The endogenic glutamic acid is catalysed by *glutamine synthase* to glutamine, the most abundant, resourceful AA in the human body [47,48]. Glutamine is involved in proper immune function, lymphocyte proliferation and cytokine production, and energy production [49,50]. The second most abundant AA in this study was leucine, at concentrations of 1.0 g/100 g, 1.0 g/100 g, 1.1 g/100 g in the RI, CB, and OPB, respectively (Table 3).

Other studies have reported on the abundance of leucine in *umqombothi* [3,23,40]. Leucine is an essential component of protein synthesis and ATP generation [51]. The concentration of proline was 0.7 g/100 g in all the samples, while its hydroxy derivative, HO-proline was not detected in any of the samples. The OPB samples had a higher concentration of essential AAs histidine, leucine, and valine compared to the RI and CB. A similar trend was observed for nonessential AAs alanine, aspartic acid, and glutamic acid. A higher AA concentration in the OPB was expected since the beer had a higher protein concentration.

Table 3. Amino acid profile of *umqombothi*.

Amino acid (g/100 g)	RI	CB	OPB
Essential			
Histidine	0.3 ^{a} ± 0.1	0.4 ^{b} ± 0.1	0.5 ^{c} ± 0.0
Isoleucine	0.3 ^{a} ± 0.0	0.3 ^{a} ± 0.0	0.3 ^{a} ± 0.0
Leucine	1.0 ^{a} ± 0.1	1.0 ^{a} ± 0.0	1.1 ^{b} ± 0.0
Lysine	0.2 ^{a} ± 0.0	0.2 ^{a} ± 0.0	0.2 ^{a} ± 0.1
Methionine	0.1 ^{a} ± 0.0	0.2 ^{b} ± 0.0	0.1 ^{a} ± 0.0
Phenylalanine	0.4 ^{a} ± 0.0	0.4 ^{a} ± 0.1	0.4 ^{a} ± 0.0
Threonine	0.3 ^{a} ± 0.0	0.3 ^{a} ± 0.0	0.3 ^{a} ± 0.0
Valine	0.4 ^{a}± 0.0	0.4 ^{a} ± 0.0	0.5 ^{b} ± 0.1
Nonessential			
Alanine	0.6 ^{a} ± 0.0	0.6 ^{a} ± 0.1	0.7 ^{b} ± 0.1
Arginine	0.4 ^{a} ± 0.0	0.4 ^{a} ± 0.0	0.4 ^{a} ± 0.0
Aspartic acid	0.5 ^{a} ± 0.0	0.5 ^{a} ± 0.1	0.6 ^{b} ± 0.0
Glutamic acid	1.5 ^{a} ± 0.0	1.5 ^{a} ± 0.0	1.6 ^{b} ± 0.0
Glycine	0.6 ^{b} ± 0.1	0.3 ^{a} ± 0.0	0.3 ^{a} ± 0.0
HO-proline	ND	ND	ND
Proline	0.7 ^{a} ± 0.0	0.7 ^{a} ± 0.0	0.7 ^{a} ± 0.0
Serine	0.4 ^{a} ± 0.0	0.4 ^{a} ± 0.1	0.4 ^{a} ± 0.1
Tyrosine	0.3 ^{a} ± 0.1	0.3 ^{a} ± 0.0	0.3 ^{a} ± 0.1
Total AAs	8.0	7.9	8.4

g—gram; HO—hydroxy; RI—mixed raw ingredients; CB—customary; OPB—optimised beer brew, ND—not detected. Mean values with different superscripts within the same row are significantly different at ($p < 0.05$).

3.4. B-Group Vitamins

The presence of B group vitamins in *umqombothi* has been widely reported [3,5,8,52]. A majority of B-group vitamins were present in all the samples (Table 4). Riboflavin (B_2), pantothenic acid (B_5), biotin (B_7), and cyanocobalamin (B_{12}) were not detected. Of all the samples, RI contained the highest concentration of all the vitamins, with the highest concentration of folic acid (B_9) (20.0 µg/g) (Table 4). Folic acid (B_9) is essential in disease prevention, neural tube defects prevention, and the methylation of homocysteine to form methionine [53–55].

Table 4. B-group vitamin content in *umqombothi*.

Vitamin (µg/g)	RI	CB	OPB
Thiamine (B_1)	0.6 ^{b} ± 0.1	0.3 ^{a} ± 0.1	0.3 ^{a} ± 0.0
Riboflavin (B_2)	ND	ND	ND
Nicotinamide (B_3)	2.7 ^{c} ± 0.0	0.1 ^{a} ± 0.0	0.2 ^{b} ± 0.0
Nicotinic acid (B_3)	2.0 ^{c} ± 0.8	0.5 ^{a} ± 0.2	0.7 ^{b} ± 0.0
Pantothenic acid (B_5)	ND	ND	ND
Pyridoxine hydrochloride (B_6)	0.1 ^{b} ± 0.0	0.03 ^{a} ± 0.0	0.0 ^{a} ± 0.0
Biotin (B_7)	ND	ND	ND
Folic acid (B_9)	20.0 ^{b} ± 2.3	0.6 ^{a} ± 0.1	0.6 ^{a} ± 0.1
Cyanocobalamin (B_{12})	ND	ND	ND

g—gram; ND—not detected; RI—mixed raw ingredients; CB—customary beer brew; OPB—optimised beer brew. Mean values with different superscripts within the same row are significantly different at ($p < 0.05$).

Even though the OPB had slightly higher vitamin concentrations than the CB, a significant difference was only observed for the isomer nicotinamide (B_3). Nicotinamide (B_3) is essential for carbohydrate metabolism and non-redox adenosine diphosphate-ribose transfer reactions involved in DNA repair [56]. Thiamine (B_1) is heat-sensitive, hence excessive heating or boiling could have led to low concentrations [57]. In addition, thiamine (B_1) and its vitamer, thiamine diphosphate are often rapidly utilised by yeasts and removed from the beer [58]. In the absence of thiamine (B_1), yeast metabolism can be sluggish, potentially leading to 'stuck' or poor-quality fermentations [59,60].

3.5. Sugars

In all three samples, maltose had the highest concentration, followed by glucose (Figure 1). It is not uncommon for maltose to be left unfermented at the end of primary fermentation [61]. Maltose metabolism depends on the yeast strain used [62], though in this study it was through natural fermentation. Unfermented maltose may lower process efficiency including irreproducible or stuck fermentations, as well as promote inconsistent yeast deposit stability and possibly influence the relative production of flavour by-products [61,62].

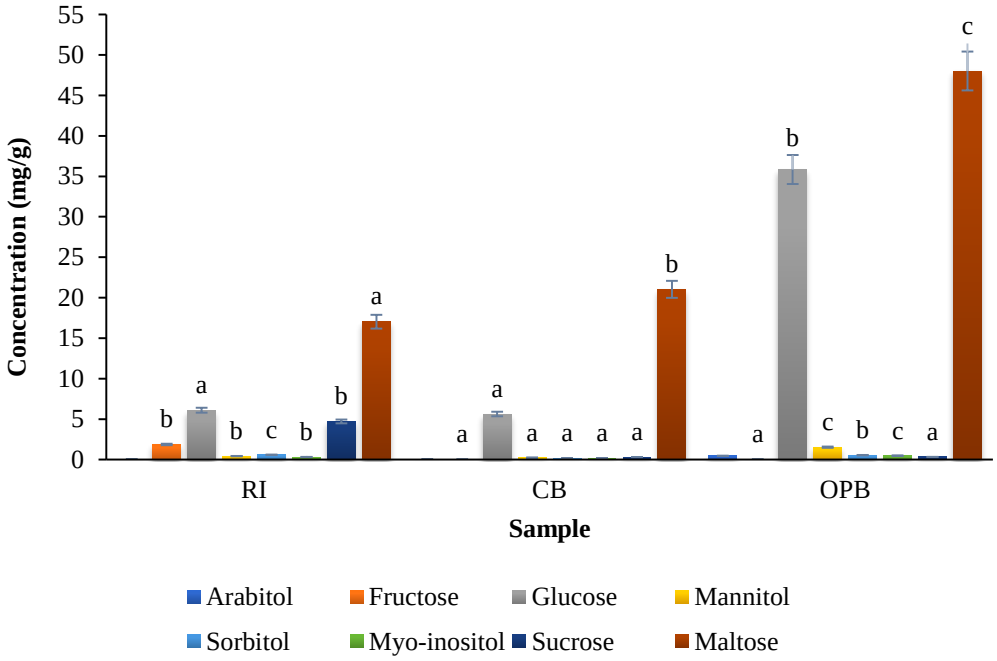

Figure 1. Sugar compounds in the mixed raw ingredients (RI), customary beer brew (CB), and optimised beer brew (OPB). Means with no common letter in the bar chart, significantly ($p \leq 0.05$) differ.

Fructose present in the RI was completely used up in both the CB and OPB (Figure 1). Similarly, the sucrose level was 4.7 mg/g, 0.3 mg/g, and 0.3 mg/g in the RI, CB, and OPB, respectively. The fermenting microorganisms may have used the principal sugars in sequential order, starting with monosaccharides such as fructose and followed by disaccharides such as sucrose [61]. Even though maltose is an abundant sugar, it is typically only be taken up after monosaccharides have been depleted [63]. A build-up of mannitol was observed in the OPB (Figure 1). At high temperatures and high concentrations of fructose or sucrose, mannitol may be produced by yeasts, bacteria, or fungi [64,65]. Mannitol is an antioxidant and can be used as a nonmetabolizable sweetener for diabetics [66].

4. Conclusions

Compared to the other samples, the OPB was relatively higher in energy (165 kcal/100 g), crude protein (8.6%), and ash content (1.0%). All the samples were relatively high in essential macrominerals and low on potentially toxic elements. Glutamic acid was the highest

detected amino acid, with concentrations of 1.5 g/100 g, 1.5 g/100 g, and 1.6 g/100 g in the RI, CB, and OPB, respectively. The OPB contained a higher concentration of the two forms of vitamin B_3, nicotinamide (0.2 µg/g) and nicotinic acid (0.7 µg/g) in comparison to the CB. The concentration of mannitol was 0.4 mg/g, 0.2 mg/g, and 2.0 mg/g in the RI, CB, and OPB, respectively, and OPB had the highest total amino acid levels. Overall, OPB displayed a desirable nutritional profile compared to the CB.

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