1

The Role of Microorganisms in the Fermentation of *Agbelima* Cassava Dough

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Abstract

The fermentation of cassava mash with a traditional inoculum into the widely consumed agbelima in Ghana, accomplishes four main objectives; a breakdown of the coarse texture, a souring of the dough, reduction in the content of cyanogenic glucosides and production of volatile aroma compounds. In all types of inocula examined, the breakdown of cassava texture occurs through the hydrolysis of cassava tuber cellulose by cellulases produced by some dominating microbial species in the inoculum. *Bacillus subtilis* and other *Bacillus* spp. including *B. mycoides*, *B. pumilus*, *B. cereus*, *B. amyloliquefaciens* and *B. licheniformis* are the tissue degrading microbial agents in three types of inocula examined and the moulds, *P. nodulum, P. citrinum* and *Geotrichum candidum* in a forth type of inoculum whose biota is dominated by moulds. The souring of agbelima is accomplished through the production of lactic and acetic acids by *Lactobacillus plantarum* and other lactic acid bacteria including *Lactobacillus brevis* and *Leuconostoc mesenteroides* in all agbelima fermentations examined. Yeasts dominated by *Candida krusei* and also including *Candida tropicalis* and a *Zygosaccharomyces* spp. together with the above mentioned lactic acid bacteria are responsible for the production of volatile aroma compounds during agbelima fermentation. The major compounds produced are a non identified low molecular weight alcohol, 1-propanol, isoamylalcohol, ethylacetate, 3-methyl-1-butanol and acetoin. Substantial reductions occur in the levels of cyanogenic compounds present in cassava during agbelima fermentation and is enhanced through the use of traditional inoculum. The lactic acid bacteria and moulds isolated from agbelima all produce significant levels of the enzyme linamarase determined as ß-glucosidase which is capable of breaking down the cyanogenic glucosides.

Keywords: Cassava fermentation; *agbelima;* bacteria; yeast; moulds; detoxification; product characteristics

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important sources of food in Sub-Saharan Africa and currently is the most widely cultivated root crop in Ghana. The total annual production in 1995 was about 6.611 million metric tonnes contributing 22% of the Agricultural Gross Domestic Product, 19% of dietary energy intake and averaging 380 cal/day per person (Dosoo and Amoa-Awua 1992; Ministry of Agriculture, Ghana 1995).

One of the principal forms in which cassava is consumed in Ghana is as a stiff porridge prepared from a sour tasting fermented cassava meal, *agbelima*. *Agbelima* is prepared by grating peeled cassava tubers together with a cassava dough inoculum, called *kudeme*, in a motorized cassava grater. The inoculated grated mash is allowed to ferment for 2 to 4 d after it has been packed tightly in polythene sacks. The product which is partially dewatered during fermentation is usually mixed with fermented maize meal and cooked into either *banku* or *akple* and eaten with stew or soup.

The processing of cassava into *agbelima* is carried out commercially as a small-scale food operation by traditional cassava processors who rely mostly on family labour to carry out the home-based operations. The product is marketed as a wet stiff dough in an active state of fermentation in the local markets. *Agbelima* production undoubtedly constitutes one of the most important industries in the traditional food sector in Ghana and serves as a means of livelihood for a number of rural women.

The current authors have investigated the microbiology and enzymatic activities of *agbelima* fermentation including four different types of cassava dough inoculum. This presentation reviews the role of the different types of microorganisms in *agbelima* production.

Agbelima fermentation

Four objectives are accomplished when grated cassava is fermented with an inoculum into *agbelima*. (I) The coarse texture of grated cassava is broken down into a smooth textured dough, (ii) acidification of the meal occurs leading to a souring of the product, (iii) there is enhanced detoxification of cassava and (iv) the dough develops a characteristic flavour (Amoa-Awua and Jakobsen 1995; Amoa-Awua 1996; Amoa-Awua *et al.*1996; Amoa-Awua *et al.* 1997).

Break down of cassava texture

The primary reason why traditional cassava processors use inoculum to ferment cassava during *agbelima* production is that the added inoculum has the ability to break down the texture of cassava dough yielding a smooth textured dough. This claim by traditional cassava processors has been confirmed by Sefa-Dedeh (1989) who found that the particle sizes of market samples of *agbelima* produced with inoculum were finer than the particle sizes of laboratory samples of *agbelima* produced without inoculum. However neither the microbiological nor biochemical agents responsible for the breakdown of cassava dough texture had been elucidated.

The results of our studies showed that the mechanism of cassava tissue breakdown during *agbelima* fermentation involves the hydrolysis of cassava tuber cellulose by cellulases produced by microorganisms present in the inoculum. This was conclusively demonstrated when (I) sterile cassava cubes incubated in cellulase solution were completely dissolved after 48 h, (ii) when the dominant microfora of four types of cassava dough inocula were shown to be cellulolytic (iii) and when these cellulolytic microorganisms were able to dissolve sterile cassava slices when they were plated directly onto the tissue (Amoa-Awua and Jakobsen 1995; Amoa-Awua *et al* .1997).

Acidification/souring of cassava dough

The characteristic image of *agbelima* is that of a sour dough similar to the spontaneously fermented cassava dough used in *gari* production. Several workers have observed the spontaneous anaerobic fermentation of grated cassava during gari production to be a lactic acid fermentation (Okafor 1977; Ngaba and Lee 1979). The microbial species responsible for the souring of inoculated cassava dough during *agbelima* production had not been identified. Our work confirmed the souring of *agbelima* to be a process of acidification as reported by previous workers (Dzeidzoave 1989; Budu 1990; Sefa-Dedeh 1994).

The pH of *agbelima* decreased from between 5.8-5.2 to 4.3-4.0 in 48 h of fermentation. The corresponding increase in titratable acidity expressed as percentage lactic acid on dry weight basis was from 0.1-0.3 to 0.8-0.9 %. These values were observed for cassava dough fermented with four different types of inocula (Amoa-Awua *et al.* 1996)

Detoxification of cassava

Detoxification of cassava is more pronounced when cassava dough is fermented with cassava dough inoculum than when it is allowed to ferment spontaneously. The enhanced detoxification was attributable to ability of the inoculum to break down cassava tissue facilitating more intimate contact between endogenous linamarase and the cyanogenic glucosides(Amoa-Awua and Jakobsen, 1995).

Development of aroma

The aroma of *agbelima* was determined in these studies to be due to the production of organic acids mainly lactic and acetic acids and also to the production of volatile aroma compounds including mainly 1-propanol, isoamyl alcohol, ethyl acetate, 3-methyl-1-butanol, acetoin and a non-identified low molecular weight alcohol during cassava dough fermentation (Amoa-Awua 1996; Amoa-Awua *et al.*1996).

The role of *Bacillus* species in *Agbelima* fermentation

The microflora of two types of cassava dough inoculum used to ferment *agbelima* were dominated *by Bacillus* spp. which appeared as rods bearing phase bright spores under the microscope. These inocula were prepared by fermenting chunks of cassava after a heat pretreatment blanching or roasting. The initial heat treatment apparently served as a means of spore activation and selection for *Bacillus* species since their heat resistant spores survived the treatment whilst most vegetative cells were destroyed. A third type of inoculum which was only briefly exposed to the warm sun but fermented in the same fashion as the blanched and roasted inocula, consistently showed much lower counts of *Bacillus* species. The *Bacillus* population in the blanched and roasted inocula were in the order of 10^7 to 10^8 cfu/g whilst in the third type of inoculum they were present at levels of 10^6 cfu/g. The *Bacillus* species introduced into cassava dough through the use of inoculum persisted throughout the subsequent *agbelima* fermentation but there was a tenfold reduction in their numbers by the end of 48 h fermentation from 10^6 to about 10^5 cfu/g (Amoa-Awua and Jakobsen 1995).

Most *Bacillus* cultures isolated from inocula and fermenting dough produced acid from D-glucose, L-arabinose, D-xylose and D-mannitol, hydrolysed casein, starch and gelatin, reduced nitrate, grew at pH 5.7 and 6.8, and in 7% NaCl, produced acetyl methyl carbinol from Voges-Proskauer medium and failed to produce indoles. In addition to the above characteristics, the most frequently isolated cultures had colonies with irregular margins and rough ridged or ringed surfaces, often producing exudate. They had small cells with ellipsoidal spores, showed urease activity and were identified as strains of *Bacillus subtilis*. These *B. subtilis* strains generally utilized ribose, α methyl-D-glucoside, amygdalin, esculin, salicin, L-arabinose, cellobiose, maltose, saccharose, trehalose, inuline, D-raffinose, starch, glycogen, glycerol, D-xylose, D-glucose, D-fructose, D-mannose, inositol, mannitol, sorbitol,

arbutin, D-turanose, melibiose and ß gentiobiose in API 50 CHB galleries. Other *Bacillus* species identified with API 50 CH were *B. pumilus, B. licheniformis, B. cereus, B. polymyxa, B. amyloliquefaciens* and *B. mycoides* (Amoa-Awua and Jakobsen 1995; Amoa-Awua 1996).

Breakdown of cassava tissue

The *Bacillus* species were examined for the production of tissue degrading enzymes, polygalacturonase, pectin esterase, cellulase and amylase and also plated directly onto sterile cassava slices. The tissue degrading enzymes had been associated by Padmaja and Balagopal (1985) with the postharvest degradation of cassava tissue. All *Bacillus* isolates exhibited cellulase activity and were also able to disintegrate cassava tissue. *B cereus, B. polymyxa* and *B. subtilis* exhibited polygalacturonase activity. As mentioned earlier, it had been demonstrated that sterile cassava cubes incubated in cellulase solution had dissolved after 48 h. The *Bacillus* spp. therefore through their cellulase activity apparently brought about the modification of cassava texture when they were introduced into *agbelima* fermentation through the use of inoculum.

Detoxification of cassava

Bacillus spp. were examined for the breakdown of 2-naphthyl- D-glucopyranoside which required ß glucosidase activity. A positive result was interpreted generally as an indication of linamarase activity even though linamarases are ß glucosidase enzymes which are substrate specific for the cyanogenic glucosides linamarin. Of all the occurring *Bacillus* spp. in *agbelima* *B. pumilus* and *B. amyloliquefaciens* showed only weak ß glucosidase activity. The other species present in cassava dough inocula tested negative. It was however concluded that even though *Bacillus* spp. could not play a direct role in the breakdown of the cyanogenic glucosides they contributed to cassava detoxification by their ability to breakdown cassava tissue. Such tissue degradation resulted in more intimate contact between endogenous cassava linamarase and the cyanogenic glucosides thus facilitating detoxification.

The role of moulds in *Agbelima* fermentation

A fourth type of cassava dough inoculum examined was prepared by peeling small chunks of cassava and superficially drying them in the open air for about 6 h (ambient temperature 30°C). The surface dried products were stuck within the thatch roof of a hut for 2 d to ferment into inoculum. The mature inoculum usually had softened tissue and was covered completely by dark mycelial growth. The fungal mycelium is carefully scrapped off and the inoculum washed with water before it is used to ferment *agbelima*.

Examination of the microbial population of this type of inoculum, the thatch inoculum, showed that it was dominated by moulds. The mould populations were usually in the order of 10^5 cfu/g but this represented the level after cleaning of the inoculum to get rid of the mycelium covering the product. Several different types of moulds were isolated in samples of thatch inoculum and fermenting dough inoculated with this type of inoculum. In the fermenting dough the moulds were usually present during the first 24 h of fermentation but most species were not isolated in the final product.

Penicillium sclerotiorum was identified as the most frequently occurring mould and had green colonies with a white border and yellow colony reverse with a red or yellowish red core when grown on Czapek yeast autolysate agar. These colonies attained a diameter of about 37mm after 7 days incubation at 25°C in complete darkness and produced the secondary metabolites sclerotiorin, rotiorin and 7-epi-sclerotiorin. *Penicillium sclerotiorum* was present in the inoculum at a level of 10^5 cfu/g and at a level of 10^4 cfu/g at 0 and 24 h of fermentation. In the final fermented product it was not isolated or present at less than 10^2 cfu/g. Other moulds identified were *Penicillium nodulum* which was present at a level of 10^4 cfu/g in both the inoculum and during the first 24 h of dough fermentation. In the final product it was not

detected at a level of 10^2 cfu/g. *Penicillium citrinum* was present in the inoculum at 5 x 10^4 cfu/g. This mould was detected at a level of 10^4 throughout 48 h of inoculated dough fermentation and was the only mould detected in the final product. *Geotrichum candidum* was present in the inoculum at a level of 10^4 cfu/g and also at the start of dough fermentation. It was not detected at 24 h of fermentation. A mould tentatively identified as a basidiomycetes was present at a level of less than 10^4 cfu/g in the thatch inoculum and also during the first 24 h of fermentation. A *Rhizopus* spp. was isolated in very low numbers in the thatch inoculum but not in the inoculated fermenting dough. *Aspergillus flavus* was isolated in one out of the three samples of thatch inoculum examined (Amoa-Awua 1996; Amoa-Awua *et al.* 1996).

Breakdown of cassava tissue

All the moulds species mentioned above were able to hydrolyse cassava tissue when plated directly unto sterile cassava slices. When tested for production of tissue degrading enzymes they all showed cellulase activity but no polygalacturonase or pectin esterase activity with the exception of a *Rhizopus* spp. which produced pectin esterase. It was concluded that moulds were responsible for breaking down cassava tissue when the thatch inoculum was used to ferment cassava dough in *agbelima* production. This was also achieved through the cellulase activity (Amoa-Awua 1996; Amoa-Awua *et al.* 1996).

The role of yeasts in *Agbelima* fermentation

Yeasts occurred in significant numbers in all four types of traditional cassava dough inoculum examined. They were also present in significant numbers in fermenting cassava dough inoculated with any of the four types of inoculum. In all inocula they were present at levels of 10^6-10^7 cfu/g. In the different types of fermenting dough their numbers increased to the same level by the end of 48 h fermentation.

Three main types of yeasts were isolated from the different inocula and fermenting dough. The most frequently isolated yeasts were characterized by being flat, off-white to greyish colonies with irregular margin fringed with pseudomycelium on Malt Agar utilized only glucose, N-acetyl-glucosamine and DL-lactate out of the 32 carbohydrates tested in ID 32 galleries and were identified as *Candida krusei*. The second type of yeasts utilized mainly glucose, galactose, sucrose, N-acetyl-glucosamine, cellobiose, maltose, trehalose, 2 keto-gluconate, methyl-D-glucoside, sorbitol, D-xylose, palatinose, melezitose, gluconate and esculin and were identified as *Candida tropicalis*. The third type of yeasts, *Zygosaccharomyces* spp. utilized only glucose in ID 32 galleries (Amoa-Awua 1996; Amoa-Awua *et al.* 1997).

Breakdown of cassava tissue

The most frequently isolated yeast *Candida krusei*, was not able to disintegrate cassava tissue and also did not exhibit cellulase activity. However *Candida tropicalis* and some strains of *Zygosaccharomyces* spp. showed weak cellulase activity and were able to break down cassava tissue on prolonged incubation. The yeasts could therefore play a role in the breakdown of cassava texture during *agbelima* fermentation but this was of secondary importance in comparison to the ability of *Bacillus spp.* and moulds to hydrolyse cassava tissue. All yeasts produced polygalacturonase but none produced pectin esterase (Amoa-Awua 1996; Amoa-Awua *et al.*1997).

Production of volatile aroma compound

Analysis of the aroma components of *agbelima* showed that yeasts contributed to the development of the volatile aroma of the product (Amoa-Awua *et al.* 1996). Some of the compounds detected by GC-MS analysis such as ethyl acetate and the alcohols, 2-methyl-1-butanol and 3-methyl-1-butanol have been described by Hansen *et al.* (1989) as yeast fermentation products.

The role of lactic acid bacteria in *agbelima* fermentation

The micropopulation of all types of fermenting dough examined on MRS medium incubated anaerobically were dominated by lactic acid bacteria reaching a level of about 10^9 cfu/g in 48 h of fermentation. The lactic acid bacteria also formed a part of the dominant flora of all four types of cassava dough inoculum examined (Amoa-Awua and Jakobsen 1996).

Lactic acid fermentation of *agbelima*

The isolation of lactic acid bacteria in high numbers in all four types of fermenting cassava dough as well as increases in titratable acidity determined as lactic acid demonstrated the acidification of *agbelima* to be a lactic acid fermentation.

Of the lactic acid bacteria identified in all types of fermenting *agbelima*, the facultatively heterofermentative lactobacilli, *Lactobacillus plantarum*, accounted for about half. *Leuconostoc mesenteroides* and *Lactobacillus brevis* were the other species of lactic acid bacteria which accounted for nearly the rest of the lactic acid bacteria population. Streptococci and other cocci belonging to the lactic acid bacteria group were isolated in much lower levels in the fermenting dough and their numbers reduce drastically as fermentation proceded (Amoa-Awua 1996; Amoa-Awua *et al.* 1996).

The dominant species identified as being responsible for the souring of *agbelima* agreed with the species of lactic acid bacteria identified by Ngaba and Lee (1979) and Okafor and Uzuegbu (1987) as being responsible for the spontaneous anaerobic fermentation of cassava dough.

Detoxification of cassava

All lactic acid bacteria isolated from *agbelima*, *L. plantarum, L. brevis, L. fermentum, L. salivarius* and *Leuconostoc mesenteroides* demonstrated considerable linamarase activity hence were capable of directly breaking down the cyanogenic glucosides present in cassava.

The aroma development

The sour taste of *agbelima* is undoubtedly due to the production of acids during fermentation. Since the lactic and acetic acids present in *agbelima* are produced by lactic acid bacteria, these microorganisms are directly responsible for the development of the dominant aroma of *agbelima*. In a product with a similar image, fermented maize meal, the major aroma components are reported to be lactic, acetic, butyric and propionic acids (Baningo and Muller 1972; Plahar and Leung 1982).

The presence of acetoin, 3-hydroxy-2-butanone in *agbelima* shows that lactic acid bacteria contribute to the development of the volatile aroma of *agbelima* (Amoa-Awua 1996; Amoa-Awua *et al.* 1996).

Conclusions

A diagrammatic summary of the microbiological and enzymatic activities which occur during the fermentation of cassava dough into *agbelima* has already been published and is presented in Fig 1 (Amoa-Awua *et al.* 1997).

Bacillus species mainly *Bacillus subtilis* and also *B. licheniformis, B. polymyxa, B. pumilus, B. cereus* and *B. mycoides* or the moulds *Penicillium sclerotiorum, P. nodulum, P. citrinum* and *Geotrichum candidum* are the microbiological agents responsible for breaking down cassava texture during *agbelima* fermentation. The yeasts *Candida tropicalis* and some strains of *Zygosaccharomyces* spp. also contribute to the breakdown of cassava texture. The breakdown of cassava texture occurs through the hydrolysis of cassava tuber cellulose by cellulases elaborated by the microorganisms.

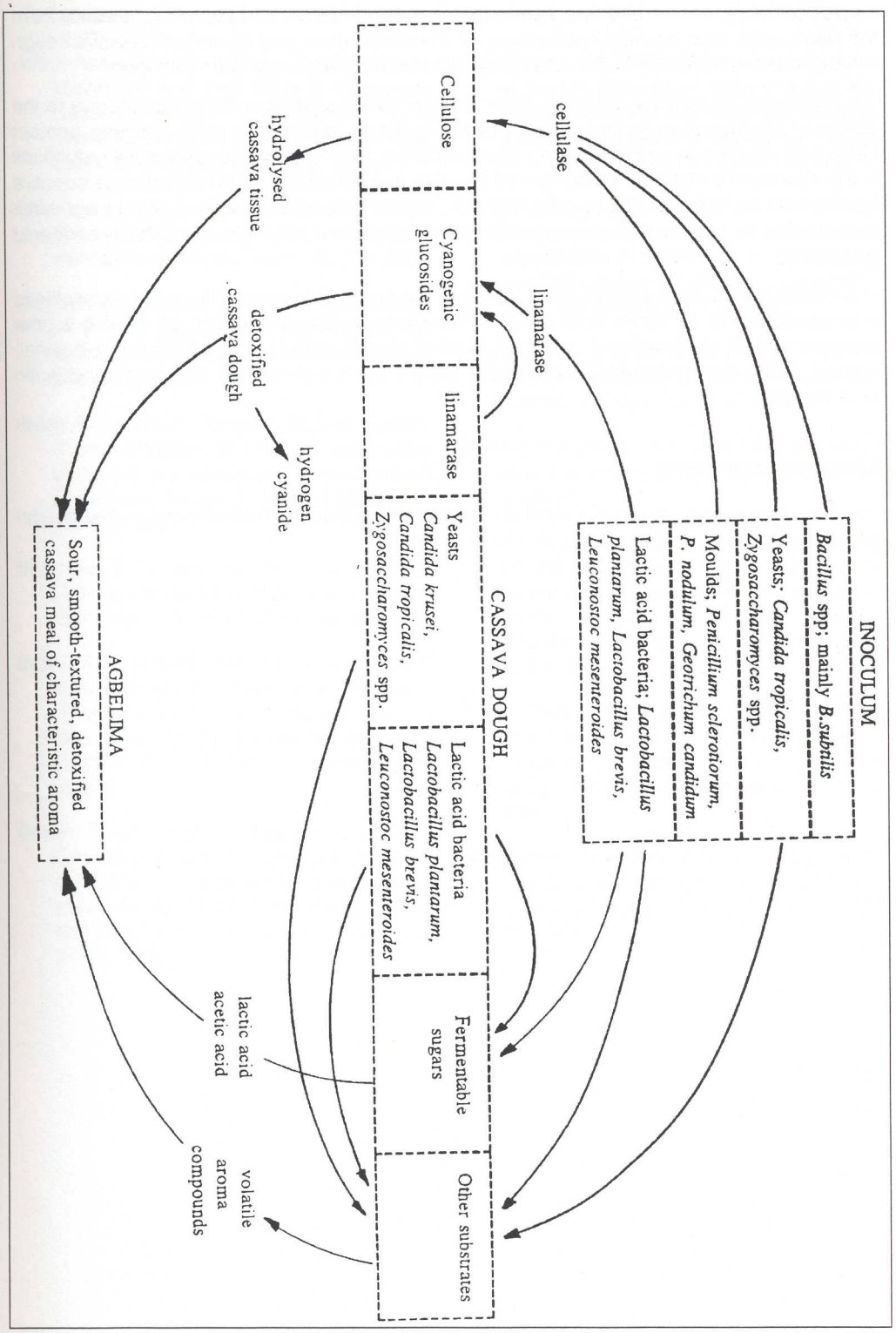

Fig. 1: A diagrammatic summary of the microbiological and enzymatic activities which occur during the fermentation of cassava dough into agbelima

Lactobacillus plantarum and also *Leuconostoc mesenteroides* and *Lactobacillus brevis* are the major lactic acid bacteria responsible for the acidification and souring of cassava dough through the production of lactic and acetic acids during *agbelima* fermentation.

Detoxification of cassava dough is more pronounced in *agbelima* fermentation due to the ability of the inoculum to break down cassava tissue resulting in more intimate contact between endogenous and the cyanogenic glucosides. Even though the substantial reductions in the cyanogenic compounds content of cassava may be attributed to endogenous cassava linamarase, the dominant lactic acid bacteria, yeasts and moulds present during *agbelima* fermentation all produced linamarase enzymes capable of breaking down the cyanogenic glucosides.

The characteristic aroma of *agbelima* is due to the production of organic acids mainly lactic and acetic acids by lactic acid bacteria and also to the production of volatile aroma compounds including mainly 1-propanol, isoamyl alcohol, ethyl acetate, 3-methyl-1-butanol, acetoin and a non-identified low molecular weight alcohol produced by yeasts and lactic acid bacteria.

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