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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Full Length Research Paper

Biopreservation of tomato paste and sauce with *Leuconostoc* spp. metabolites

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The aim of this study was to evaluate 40 lactic acid bacteria (LAB) and 20 *Bacillus* strains isolated from the fermented tomato (*Solanum lycopersicum*) for their capacity to produce antimicrobial activities against several bacteria and fungi. The strain designed LBc03 has been selected for advanced studies. The supernatant culture of this strain inhibits the growth of *Escherichia coli*, *Staphylococcus aureus* and *Aspergilus* sp. Based on the cultural, morphological, physiological and biochemical characteristics, LBc03 was identified as *Leuconeustoc* spp. Its antimicrobial compound was determined as a proteinaceous substance, but it is possible that the bacteriocin may also be bound to other molecules like a lipid or a carbohydrate moiety. Metabolite extracts from selected LAB were more effective in preserving tomato paste and sauce stored at 4°C against spoilage bacteria like *E. coli* and the application of bio-preservative should be encouraged in food processing industries.

Key words: Biopreservation, tomato (*Solanum lycopersicum*), bacteriocins, lactic acid bacteria.

INTRODUCTION

Microbial spoilage of fruits and vegetable is known as rot, which manifests as loss of texture (soft rot), changes in color (black or grey) and often off odor (Trias et al., 2008). Also, the high water content in tomatoes makes it very susceptible to spoilage bacteria and fungi during storage, harvesting and transportation (Spadaro and Gullino, 2004). Fresh food like fruits and vegetables, are normal part of the human diet and are consumed in large quantities in most countries. These products are rich in carbohydrates and poor in proteins with pH value from slightly acidic to 7.0 and provide a suitable niche to several bacteria, yeasts and moulds (Wiessinger et al., 2000; Trias et al., 2008 ; Ogunbanwo et al., 2014). Tomato (*Solanum lycopersicum*) is one of the highly nutritious food ingredient used in the preparation of food all over the world (Ogunniyi and Oladejo, 2011; Ogunbanwo et al., 2014). Its utilization as an ingredient in vegetable salads, other dishes and its processing into different products like puree, ketchups and juice is well documented. Nutritionally, it contains a large amount of water, niacin, calcium and vitamins especially A, C, E which are important in the metabolic activities of man and protects the body against diseases (Taylor, 1987). Lycopene (acarotene) an essential component of tomato contributes in the prevention of cardiovascular disease and cancer of the prostrate (Clinton, 1998; Bernard et al., 1999).

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Table 1. S01, S02, S03, and S04 tomatoes *Solanum lycopersicum* samples.

Samples	Origin	Tomato Variety	Characteristics	Additives added	Temperature of storage (°C)	Storage time
S01[C1]	Mascara, Algeria	*Solanum lycopersicum*	Fresh-cut tomato	Absence	25	24 h
S02[C2]	Mascara, Algeria	*Solanum lycopersicum*	Tomato paste was boiled for 90°C for 02 h	Absence	25	24 h
S03	Mascara, Algeria	*Solanum lycopersicum*	Fresh-cut tomato	Absence	-20	06 months
S04	Mascara, Algeria	*Solanum lycopersicum*	Tomato paste was boiled for 90°C for 02 h	05% NaCl and oil	+4	06 months

C1and C2 are control samples.

The characteristic flavor of tomato is produced by the complex interaction of the volatiles and non-volatile components (Petro-Turza, 1987; Buttery, 1993). The nutritional value of tomato products is a topic attracting much attention, particularly regarding the effects resulting from food processing and storage treatments (Capanoglu et al., 2010). Among the common post-harvest fungal pathogens of tomatoes are *Pencillium expansum*, *Monilinia laxa* and *Rhizopus stolinifer* (Ogawa et al., 1995; Pla et al., 2005). Many LAB strains are able to produce protein compounds with efficient antimicrobial effect, which are known as bacteriocins (Davidson and Harrison, 2002). In recent times, the understanding of the preservation mechanisms of LAB is being exploited for industrial production of foods (Trias et al., 2008) because of their natural acceptance as Generally Recognized as Safe (GRAS) for human consumption and exhibit antimicrobial property (Aguirre and Collins, 1993). There is a complimentary effect by the production of acid and antimicrobial compounds that increases inhibition of both pathogen and spoilage bacteria (Edwards et al., 1983; Artés et al., 1999). Although many efforts have been made to develop bio-protective lactic acid bacteria strains, the application of these strains in fresh fruits and vegetables have not been developed yet (Toivonen and DeEll, 2002).

This work is designed to investigate the effectiveness of lactic acid bacteria and *Bacillus* metabolites in preserving tomato paste and sauce against *S. aureus, E. coli*, *Clostridium* sp., *Aspergillus* sp., and *Penicillium* sp.

MATERIALS AND METHODS

Samples collection

Fresh tomato samples *Solanum lycopersicum* (Table 1) were purchased from markets in Mascara, Algeria. The samples were collected in separate sterile polythene bags and immediately transported to the laboratory for analysis.

Determination of the physical-chemical characteristics of the tomato samples (S01, S02, S03, and S04)

Moisture content

The moisture content of the samples (S01, S02, S03, and S04) was determined before storage time by weighing into moisture cans then weighed and then placed in an oven at 80°C for 24 h to dry to a constant weight. Then, brought out and allowed to cool in desiccators and then reweighed (A.O.A.C., 1995).

$$\text{Thus, moisture content} = \frac{(\text{initial weight} - \text{final weight})}{\text{initial weight}} \times 100$$

Dry matter content

Five grams of each sample were obtained and placed into pre-weighed crucibles and dried in at 100°C for 12 h. The dried samples were weighed after cooling in the desiccators (A.O.A.C., 1984).

Determination of lactic acid produced by lab isolates

This was achieved based on the methods described (Ogunbanwo et al., 2008; Bamidele et al., 2011). For these measurements lactic acid was determined by transferring 25 ml of the tomato juice (S01, S02, S03, and S04) into conical flasks and 3 drops of phenolphthalein were added as indicator. From a burette, 0.1 M NaOH was slowly added to the samples until a pink color appeared. Each ml of 0.1 M NaOH is equivalent to 90.08 mg of lactic acid.

Determination of the pH

pH was determined after and before fermentation of the tomato samples (S01, S02, S03, and S04) by the used of pH meter (Inolab MLM) (Lehninger, 1981).

Detection of pathogens, contaminants, LAB, and bacillus

The main objective of this experiment was to detect microbial contaminants in tomato samples (S01, S02, S03, and S04) in addition to other related hygiene tests. Total aerobic bacterial count,

total coliform count, Total anaerobic bacteria count, *Salmonella*, *S. aureus*, *Clostrudium*, lactique acid bacteria, and *Bacillus* were detected (Delarras et al., 2006).

Isolation and selection of LAB strains

LAB strains were isolated from the tomato samples (S01, S02, S03, and S04). The samples were plated directly on MRS as detailed by De Man, Rogosa and Sharpe (Merck, Germany) and M17 agar (Merck, Germany) at 30°C for 2-3 days (pH6.5) under aerobiosis and anaerobiosis conditions. They were routinely propagated and stored at – 20°C supplemented with glycerol (20%, v/v, final concentration). Working cultures were sub-cultured twice (1 inoculum, 24 h, 30°C) prior to use (Djadouni and Kihel, 2012; Ogunbanwo et al., 2014).

Identification of the LAB isolates

The pure isolate selected as a potential bacteriocin - producer was identified on the basis of its cultural, morphological, physiological and biochemical characteristics. The selected LAB isolates were characterized by Gram stain, absence of spores and catalase test. Gram+, catalase and spores negative strains were maintained frozen until needed for the antimicrobial activity testing. Confirmation of the identification was based on the use of Bergey's manual of systems bacteriology (Sneath et al., 1986).

Isolation and Identification of bacillus strains

Samples of tomato were weighed as 1 g portions and thoroughly homogenized insterile distilled water; serial dilutions were plated on LB agar (Luria–Bertani media, Merck, Germany) plates were incubated at 30°C for 2-3 days (Kalil et al., 2009). The pure isolate was examined macroscopically and microscopically and identified with reference to Holt et al., (1994). Isolates were identified by colonial appearance, gram positive, and presence of spores, the presence or absence of β-haemolysis, lecithinase activity, motility, penicillin susceptibility and biochemistry (Abriouel et al., 2010).

The indicator strains

The indicator strains (*S. aureus, E. coli*, *Clostriduim* sp., *Aspergillus* sp.*,* and *Penicillium* sp.) used in this work was provided by the Laboratory of Bacteriology, Microbiology Department at the Faculty of Sciences, Es-Senia, Oran University, Algeria. For the antimicrobial assay, the pathogenic cultures were grown in the nutrient agar media (NA) at pH, 7.4 (Ogunbanwo et al., 2014).

For antifungal activities determination, *Aspergillus* sp. and *Penicillium* sp. were grown in potato dextrose agar (PDA, Merck, Germany) for 7 days at 30°C. Spores were collected in sterile distilled water and then concentrated to 10^4 spores ml^{-1} (Smaoui et al., 2010).

Preparation of cell-free filtrate

MRS broth (1000 µl) were inoculated separately with isolates (LAB or *Bacillus*) previously characterized and incubated at 30؛C for 72 h. After incubation, a cell free supernatant was obtained by centrifuging (Spectrafuge 24D, Labnet, USA) the bacterial culture at 10.000 rpm for 45 min, followed by filtration of the supernatant through 0.2 mm pore size filter paper thus obtaining cell free filtrate (Khalil et al., 2009; Djadouni and Kihel, 2013).

Antagonistic activity of LAB and bacillus metabolites against spoilage microorganisms

Sixty isolates (40 LAB and 20 *Bacillus*) were grown in MRS broth for 72 h at 30°C and the broth cultures were centrifuged at 10.000 rpm for 30 min and the supernatant containing the metabolites were obtained and 100 µL of the supernatant was transferred into wells (6 mm diameter) bored in Muller Hinton and potato dextrose agar previously seeded with the spoilage bacteria cells and fungi spores. The culture plates were incubated at 30 °C for 48 h and 7 days respectively and observed for zones of inhibition (Ogunbanwo et al., 2014).

Characterization of the antimicrobial substance

The isolated crude antimicrobial substance was characterized with respect to the effect of proteolytic enzymes on the bacteriocin activity. Selected enzymes were tested on the cell free supernatant (Bizani and Brandelli, 2002). Proteolytic enzymes including trypsin, pepsin, and papain were dissolved in 40 mM Tris-HCl (pH, 8.2), 0.002 M HCl (pH, 7), and 0.05 M sodium phosphate (pH, 7.0) respectively to a final concentration of 0.1 mg ml^{-1}. Other enzymes such as lipase and α-amylase were dissolved in 0.1 M potassium phosphate (pH, 6.0), and 0.1 M potassium phosphate (pH, 7.0) respectively to a final concentration of 0.1 mg/ml. Equal aliquots of both filter sterilized of each test strain and each enzyme solution were mixed, incubated at 30°C for each enzyme for 2 h and heated in boiling water for 5 min to inactivate the enzymes. These sample mixtures and the controls (without enzyme treatment) were inoculated with the indicator strains as previously mentioned and tested for antimicrobial activity by the optical density method (ODM) (Smaoui et al., 2010; Djadouni and Kihel, 2013).

Shelf life study of tomato paste and sauce

The tomato paste and sauce were boiled for 10 min and dispensed in 20 g amount separately into three pre-sterilized containers. Twenty milliliters of the crude bacteriocin-like substance of *Leuconostoc* spp. were added (v/v) differently to the tomato paste and sauce inoculated with *E. coli* 10^7 CFU g^{-1} (2 ml) and stored at 4°C for 72 h. Microbial load of each treatment was monitored by determining the colony forming unit (CFU ml^{-1}) of *E. coli* on the hektoen medium (Safdar et al., 2010; Cottaz et al., 2008).

Statistical analysis

Data were expressed as mean ± standard deviation. Statistical significance was determined using one-way analysis of variance on the replicates, where a p-value of ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

The results of the physical and chemical analyses of tomato samples are shown in Table 2. The percentage of moisture contents decreased in S02 and S04 samples to 60.30 and 62.50%; this may be due to the boiling at 90°C for 2 h and the production process steps of tomato paste, that is made by cooking tomatoes for several hours to reduce moisture, straining them to remove the seeds and skin (Adam, 1998; Ife Fitz and Bas Kuipers, 2003; Trgera et al., 2007; Souci et al., 2008). Dry matter content was

Table 2. Physical-chemical characteristics of tomatoes samples (S01, S02, S03, and S04).

Samples	Moisture content %	Dry matter content (g)	lactic acid content (°D)	pH	Firmness, taste and color
S01	94.20 ± 0.1	5.9 ± 0.1	0.99 ± 0.02	04.20±0.03	No change
S02	60.30 ± 0.1	5.7 ± 0.2	0.75 ± 0.02	04.43±0.03	Change in color
S03	94.20 ± 0.1	5.8 ± 0.1	0.89 ± 0.02	04.28±0.02	No change
S04	62.50 ± 0.1	5.9 ± 0.1	01.58 ± 0.02	03.70±0.01	Change in color, taste, and production of CO_2

stable along the different time and temperature of storage in S01, S02, S03 and S04 from 5.9 g to 5.7 g, but the pH values decreased to 03.70 in sample S04 that was stored at 4°C for six months, and lactic acid concentration increased to 01.58 D° during this storage period with an important change in the color, taste, and CO_2 production; this changes may be due to the thermal treatment, fermentation process and organic acids (citric acid, ascorbic acid, and lactic acid) produced by LAB during storage in anaerobic conditions in presence of oil and NaCl (Jean-Louis, 2007).

The fresh-cut tomato S03 was not affected during cold storage and shelf-life. On the other hand, S01 and S02 did not show significant differences among them. Color of fresh-cut tomatoes was significantly affected by the antimicrobial treatments and the storage time (Ayala-Zavala et al., 2008). Previous experiments demonstrated that firmness losses of fresh-cut tomato slices were probably linked to the ripening stage of whole fruit at processing and storage temperature. In addition, processing operations may have triggered important losses in membrane integrity due to mechanical stressing of plant tissues. In this regard, diminishing membrane integrity could cause a loss of texture related enzymes and their substrates, leading to a rise in Xuids and solute exchanges as well as an increase in the enzymatic activity (Ayala-Zavala et al., 2008). Results obtained in this study show that transformation process, temperature and storage period can affect the tomato components and quality (Goodman et al., 2002; Chong et al., 2009; Ife Fitz and Bas Kuipers, 2003). During transport and storage, intact fruits and vegetables are prone to deleterious changes induced by respiratory, metabolic and enzymatic activities, as well as by desiccation, pests, microbial spoilage, and temperature-induced injury. Many of these changes adversely affect the antioxidant status of the tomato products (Lindley, 1998). Capanoglu et al. (2010), worked closely with a Turkish tomato paste factory and obtained samples from each step during the paste production process, from the tomatoes arriving at the factory gate to the final canned product. Both targeted (for carotenoids) and untargeted (LC-MS of semi-polar, hydrophilic extracts) metabolic approaches were used to follow biochemical changes after each step.

Results show that a multitude of modifications took place, involving both increases and decreases in individual components. Those steps causing the greatest changes were identified and predictions were made as to how these steps could be tackled in a modified processing strategy to improve the antioxidant capacity of the end product. Gahler et al. (2003) investigated home preparation methods such as peeling, tomato soup preparation, etc., and three different steps of tomato juice production including sieving, homogenization, sterilization, filling, and pasteurization. Results suggested that homogenization increased the hydrophilic antioxidant capacity of the different tomato products. However, the exact mechanism still remains unclear. Similarly, in industrial processing, Capanoglu et al. (2010) also showed the “breaker” or homogenization step most significantly altered the biochemical composition of tomato paste. Abushita et al. (2000) also analyzed samples taken from three steps of paste processing (raw tomato, crushed sieved puree, and pasteurized paste) which were obtained from a canning factory in Hungary. The results show that the contents of ascorbic acid and tocopherols decreased during processing while carotenoids either remained unchanged or were found to increase.

Absence of *Salmonella*, *S. aureus*, and *Clostridium* in the tomatoes samples (Table 3) showed that thermal treatment and cold storage of tomato inhibit the growth and developments of pathogens and spoilage micro-organisms. However, thermal treatments applied during industrial preparation of tomato products may involve various chemical reactions leading to the degradation of these antioxidants. Besides, addition of vegetable oil for the preparation of tomato sauce may lead to lipid oxidation contributing to the micro-constituent instability (Chanforan et al., 2010a and b). The presence of some aerobic mesophilic microorganisms, coliforms, and anaerobic bacteria compared to control samples was related to the averments conditions and preparations methods of tomatoes. Ayala-Zavala et al. (2008) suggest that mechanical damage during minimal processing enhance contamination by epithelial microflora, and promote leaking of nutrients, which are rich substrates to microorganisms, supporting the fast microbial development in contrast with intact tissue.

The highest count of LAB and *Bacillus* in S04 sample was explained by the storage of tomato paste for 06 month at 4°C in the presence of oil and NaCl, which favor the fermentation and organics acids production that

Table 3. Detection of pathogens, contaminants, LAB, and *Bacillus* in the tomato samples (S01, S02, S03, and S04).

Samples	Total aerobic bacterial count (CFU ml^{-1})	total coliform count (CFU ml^{-1})	Total anaerobic bacteria count (CFU ml^{-1})	*Salmonella* (CFU ml^{-1})	*Clostrudium* (CFU ml^{-1})	*S. aureus* (CFU ml^{-1})	LAB (CFU ml^{-1})	*Bacillus* (CFU ml^{-1})
S01	86.10^5	36.10^5	30.10^5	ABS	ABS	ABS	ABS	20.10^5
S02	55.10^5	17.10^5	34.10^5	ABS	ABS	ABS	20.10^5	25.10^5
S03	76.10^5	ABS	ABS	ABS	ABS	ABS	47.10^5	29.10^2
S04	ABS	25.10^5	36.10^5	ABS	ABS	ABS	60.10^6	72.10^5

* ABC, absence.

decreased the pH of products and increased also the CO_2 production; these conditions inhibit the growth of contaminants in the tomato paste (Buta and Moline, 1998; Benkeblia, 2004). Also, this LAB and *Bacillus* have antibacterial and antifungal activities against a variety of Gram-negative and Gram-positive bacteria and fungi. Related to fungal development, the highest counts were observed in control samples (Siboukeur, 2011).

A total of 60 isolates were obtained from fermented tomato paste and screened for antimicrobial spectrum against the Gram-positive, Gram-negative bacteria, and fungi using the well diffusion method. The average diameter of the inhibition zone measured ranged from 2 to 4 mm size. One strain of LAB was selected for further studies, because LBc03 contained antimicrobial compound with wide spectrum that inhibited the growth of three indicator strains *E. coli*, *S. aureus*, and *Aspergilus* sp but did not inhibited *Penicillium* sp. and *Clostridium* sp. On the basis on their positive Gram reaction, non-motility, absence of catalase activity and of spore formation, the rod or coccal shape, physiological and biochemical characters as well as sugar utilization pattern, LBc03 was identified as *Leuconostoc* spp. (Table 4). The isolation of LAB species from healthy tomato fruits is in accordance with findings of Sajur et al. (2007) and Settanni and Corsetti, (2008). The presence of LAB in tomato fruit is attributed to their high survival in post harvest conditions of tomatoes (Trias et al., 2008; Ogunbanwo et al., 2014).

The LAB ability to produce antimicrobial compounds is due the absence of true catalyses to break down hydrogen peroxide generated which accumulates and becomes inhibitory to some organisms. The antagonistic activity of LAB metabolites against the spoilage bacteria and fungi agrees with the findings of Trias et al. (2008). The inhibitory effect of lactic acid is due to undissociated forms of the acids which penetrates the pathogen's membrane and liberate hydrogen ion in the neutral cytoplasm thus inhibiting vital cell functions (Corleh and Brown, 1980; Adeniyi et al., 2006). Diacetyl is known to be very effective against fungi and this is due to the interference with the utilization of arginine (De Vyust and Vandamme, 1994) and in addition to a strong oxidizing effect on the organisms cell especially bacteria (Condon, 1987; Sangorrin et al., 2014).

The *Leuconostoc* spp. (LBc03) bacteriocin-like substance produced was inactivated by the tested enzymes (Table 5). However, since the activity of the filtrate was not completely inhibited, it is possible that the bacteriocin may also be bound to other molecules like a lipid or a carbohydrate moiety. These data clearly show that the bacteriocin-like substance was of a proteinaceous nature.

Similar results were obtained (Joshi et al., 2006; Diop et al., 2008; khalil et al., 2009; Chanforan et al., 2010a and b; Rakshita, 2011).

Bacteriocin-like substance produced by LBc03 resulted in the decrease of *E. coli* from an initial population of 10^7 to 05.10^3CFU mL^{-1} and 1.10^3 CFU mL^{-1} in tomato paste and sauce respectively (Figure 1). This reveals a possible potential of the LAB metabolites in the retardation of food spoilage which agrees with the findings of Ogunbanwo et al. (2008).

The bio-preservative potential of LAB metabolites has been tested on other food product like suya (Adesokan et al., 2008) and chicken meat (Ogunbanwo and Okanlawon, 2006). A major advantage in the use of lactic acid bacteria and their metabolites is that they are considered as generally recognized as safe (GRAS) and comply often with the recommendations for food products (Stiles and Holzapfel, 1997). Unlike some chemical preservatives, LAB metabolites have not been reported to have residual effect on the food product or the consumer's health.

Conclusion

Our bacteriocin-like substance revealed interesting properties that justifies it importance regarding food safety and protection.

Conflict of interests

The author(s) did not declare any conflict of interest.

Table 4. Morphological, physiological and biochemical properties of LAB isolate (LBc03).

Test	Isolate LBc03
Morphology	**Rod, circular and white colonies 3.0mm**
Growth at temperature (°C)	
10	+
15	+
30	+
37	+
45	+
Growth at pH	
3.5	-
4.5	+
5.5	+
6.5	+
7.5	+
8.5	+
9.5	w
Growth at NaCl %	
4	+
6.5	+
10	+
15	+
CO2 from glucose	+
CO2 from gluconate	+
ADH	+
Citrate	+
Thermoresistance at 60°C for 30 min at 45°C	+
Fermentation Type	He
Fermentation of: glucose, saccharose, and lactose.	+
Lait Sherman (1% BM) at 42°C	+
Lait Sherman (3% BM) at 42°C	-
Hydrolyse of caseine	+
Fructose	+
Mannane	+
Maltose	+
Trehalose	+
Manose	+
Melibiose	-
Palmitine	-
Raffinose	+
Xylose	-
Mannitol	+

+, Growth; -, no growth; w, weak growth; He, hetero-fermentation.

Table 5. Effect of enzymes treatment (1 mg/mL) on bacteriocin activity against *S. aureus*. Results are expressed as % of means values of growth reduction (n= 3) ± standard deviations.

Enzymes	Enzyme concentration (1 mg mL^{-1})
Pepsin	38.50 ± 0.5
Trypsin	40.30 ± 0.5
α- Amylase	41.80 ± 0.3
Lipase	40.80 ± 0.5

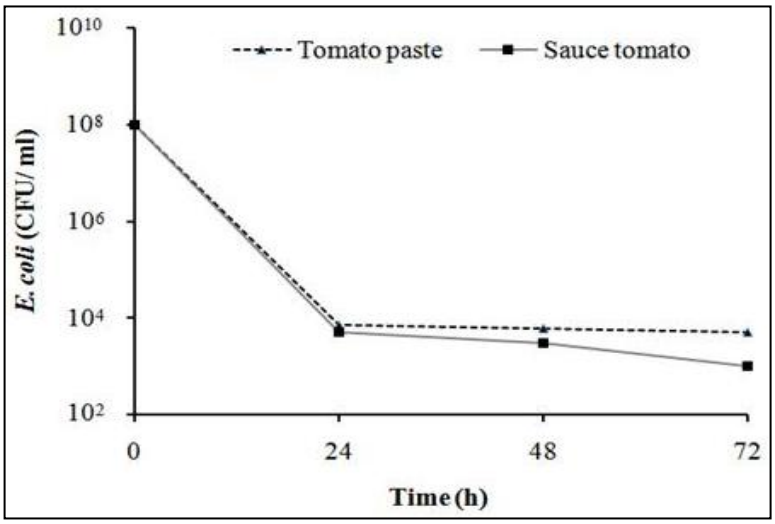

Figure 1. Reduction of *E. coli* population in paste and sauce tomato treated with natural antimicrobial of LBc03 isolate and stored at 4°C for 03 days.

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Full Length Research Paper

Screening of antibiotics residues in beef consumed in Ouagadougou, Burkina Faso

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The anarchic use of antibiotics for therapeutic purposes or as a growth factor in Burkina Faso's breeding is the origin of their residues present in consumed meat. These antibiotics residues have health, technological and microbiological consequences. The objective of this first study is to highlight the antibiotic residues in meat consumed in Ouagadougou, Burkina Faso. A survey was carried out to make an inventory of antibiotics used for animal treatment intended for production and the consequences of such use. Thus, a total of 100 samples of bovine kidney were deducted from the refrigerating slaughter house of Ouagadougou aseptically to test the presence of drug residues with the Premi®Test kit and the microbiological method with *Geobacillus stearothermophilus* var. calidolactis ATCC 10149. According to this analysis, 31% of the kidney samples contained aminoglycosides, quinolones, macrolides and beta-lactam, sulfonamides and/or tetracyclines causing the zones of inhibition 3 to 15 mm. This reflects the anarchic use of antibiotics in Burkina Faso cattle breeding. Measures must be taken to ensure consumer safety and reduce the impact of these antimicrobials on selection of resistant pathogenic bacteria strains.

Key words: Consumed meat, antibiotics residues, Burkina Faso.

INTRODUCTION

Foodborne diseases caused by microbial agents, biotoxins and chemical pollutants constitute a serious public health problem (FAO/WHO, 2004). Among the chemical pollutants found in food, antibiotics residues occupy a prominent place (votimir et al., 2011). Serious foodborne infections of epidemiological proportions have been reported globally in the past decades, showing their importance both for public health and social plan. Worldwide, consumers are increasingly concerned about these epidemics such as bovine spongiform encephalopathy, avian influenza etc. (Lantier et al., 2004). The anarchic (lawless) use of antibiotics for therapeutic purposes or as a growth promoter in lives in livestock is causing serious problems associated with the presence of these residues in food

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animals such as milk, meat, eggs and fish (Catery et al., 2003; Ben-Madhi and Ouslimani, 2009).

Antibiotic residues in animal source foods are responsible for the modification of the intestinal flora (Abidi, 2004; Chestnut and Stevens, 2005), allergies by beta-lactams (Fabre and Joye, 2000a; Fabre et al., 2000b) and antibiotic resistance (Gysi, 2006). These residues constitute an important source of potential toxicological risk to the consumer (Kabir et al., 2004; Persoons, 2011). Besides these health risks, the antibiotic residues in milk and meat are responsible for the inhibition of fermentation processes (Heeschen and Blüthgen, 1990). Indeed, fermentative bacteria are often inhibited by low dose of antibiotics which raises manufacturing accidents (absence of fermentation) (Oliveira et al., 2006). These accidents affect industries and manufacturing units of dairy and meat processing, causing huge economic losses (Brouillet, 2002; Oliveira et al., 2006). In most of West Africa countries and Burkina Faso in particular, misuse of antibiotics by farmers have been reported (Biagui, 2002). Burkina Faso has an important bovine livestock in perpetual growth, 8,233,845 heads of bovine in 2009 (MRA, 2009).

Antibiotics and other veterinary drugs are given anarchically to animals for therapeutic, prophylactic purposes, and as growth promoters. This misuse has led to a proliferation of illicit drugs and self-medication among farmers (MRA, 2009). Faced with this misuse of veterinary drugs and the lack of information about antibiotics in meat residues in Burkina Faso, this work was aimed at detecting the antibiotic residues in the meat consumed in Ouagadougou, Burkina Faso.

MATERIALS AND METHODS

Investigation of the use of veterinary drugs

In order to make an inventory on the use of antibiotics, investigation and census were carried out from May to July, 2012 at the "Direction Générale des Services Vétérinaires (DGSV)" of Burkina Faso, veterinary pharmacies and clinics and nearby breeders. Specific questions were asked about the recording mechanisms of veterinary drugs, the place of antibiotics in veterinary drugs registered, the place of tetracycline in antibiotics, antibiotics largely sold. In breeding, tetracycline usage is important about the breeders (MRA, 2011). There was also a point about the practice of self-medication by breeders, knowledge of the timeout and respect of that period.

Sampling

This study focused on oxen kidneys slaughtered in the refrigerating slaughter house in Ouagadougou, Burkina Faso from March to July 2012. Kidneys are routinely used for detection of antibiotic residues (tetracycline/oxytetracycline, enrofloxacin, ciprofloxacin) in beef as they allow an overall assessment of the presence of antimicrobial residues in all carcass (Cooper et al., 1998; Myllyniemi et al., 2000; Cantwell and O'keeffe, 2006). A total of 100 samples were collected immediately after slaughter, the sampling was done with a frequency of 7 kidneys week. The oxen's carcasses' were destined to human consumption. An amount of 230 to 380 g of kidney were removed, collected in sterile bags and placed in an isothermal box at 4°C before being transported to the laboratory for freezing at -20°C and analysis.

Screening of antibiotic residues

The frozen samples were thawed immediately and the liquid therein was recovered for analysis. Two successive tests were conducted to check the effect of lysozyme on the detection of antibiotic residues. The exudation (liquid) from the kidney contains active lysozyme capable to inhibit the growth of microorganism (Pikkemaat, 2009; Merten, 2010). The first test was performed with the active lysozyme and the second with the inactive lysozyme. A volume of 1 ml of liquid from the kidney was heated at 75°C for 15 min to inactivate the lysozyme (Nouws, 2000). The samples underwent firstly an initial screening with the Premi® Test Kit (DSM Premi® Test, NETHERLANDS). Premi® Test is qualitative and can detect the family of beta-lactam antibiotics, sulfonamides, amino-glycosides, quinolones, macrolides and tetracyclines. Samples found positive in comparison to positive control using the test kit were subjected to the confirmation by the microbiological method (AFSSA, 2006) with *Geobacillus stearothermophilus* ATCC 10149. In fact Mueller Hinton Agar (Liofilchem Italy) was first sowed by *G. stearothermophilus* to the order of 10^5 spores/ml. This suspension was prepared by pure colonies emulsion in physiological saline (NaCl: 9 g/L of water) and adjusting the absorbance in a spectrophotometer at 625 nm to a value of between 0.08 and 0.1 (equivalent to 0.5 McFarland standard). Sterile Wattman paper disks impregnated with the liquid kidney to undergo a capillary test were deposited on the surface of the inoculated agar and incubated at 55°C. After 24 h of incubation, the diameters of the zone of inhibition were measured. While diameters of the zone of inhibition was greater than 3 mm, the samples were considered positive and the diameters were low at 1 mm; as they were negative about positive and negative control. Targeted antibiotics are originated from the family penicillin and tetracycline.

DATA ANALYSIS

Data were entered and analyzed with EXCEL 2003® and WIN EPISCOPE® 2.0. Software. The frequencies were calculated with a confidence interval (CI) of 95% and a margin of error of 5% (0.05).

RESULTS

Survey

Veterinary drugs' Market authorization (MA) in Burkina Faso

It is clear from this survey that the veterinary drugs are subject to the Market authorization (MA) in Burkina Faso. The registration process takes into account aspects such as specialty of pharmaceutical form, dosage and presentation.

Veterinary drugs sold in Burkina Faso

Among the veterinary drugs sold in Burkina Faso, antibiotics occupy an important place. Table 1 show that

Table 1. Distribution of veterinary drugs in Burkina Faso in 2009 (MRA, 2009)

Veterinary drugs	Quantity in ton and percentage
Antibiotics	2686, 95 (35%)
Antiparasitic	2456, 64 (32%)
Anti-inflammatory	383, 85 (5%)
Vitamins and others	2149, 56 (28%)
Total	7677 (100%)

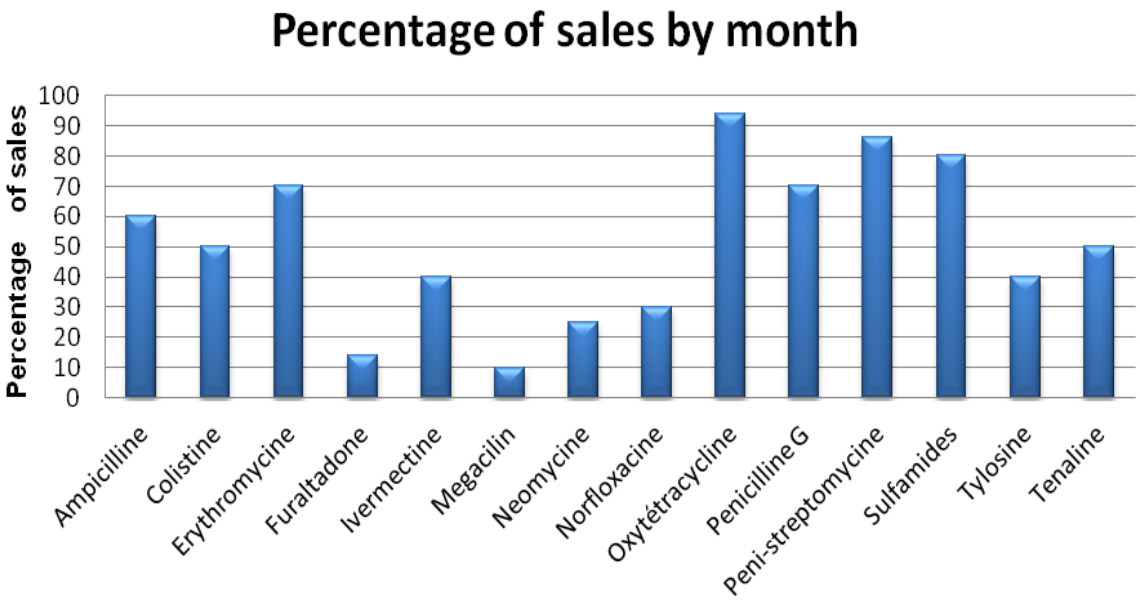

Figure 1. Main antibiotics sold in pharmacies and veterinary clinics and their monthly sales proportion in Ouagadougou.

antibiotics account for 35% of the total quantity of veterinary drugs in Burkina Faso in 2009.

Antibiotics and sales proportions

Figure 1 shows the different antibiotics, proportions of sales and that commercialized. Oxytetracycline were most 94% sold followed by penicillin-streptomycin and the less (10%) used is megacilin.

Timeout's knowledge of breeders

No information and recommendation regarding the timeout are given to breeders who go to veterinary pharmacies to buy antibiotics. Regarding the good farming practices, among the 50 farmers who were interviewed 70% (35/50) said that they do not refer to a vet before treating their animals. 31% (11/50) of breeders admit to inject their animals.

Table 2. Prevalence of antibiotics residues without lysozyme inactivation.

Methods	Premi® test	Microbiological test
Positive	56 (56%)	59 (59%) [3 to 16mm]
Negative	44 (44%)	41(41%) [< to 3mm]

n= Number of samples; ( ) = Percentage; [ ] = Minimum and maximum diameters of inhibitions zones measured.

Antibiotics residues

Microbiological and Premi® tests results without lysozyme inactivation shown respectively that 59 and 56% of samples were positive (samples that have caused zone of inhibition been greater than or equal to 3 mm for beta-lactam residues and/or sulfonamides, aminoglycosides, quinolones, macrolide and/or tetracyclines) (Table 2). Also, after lysozyme inactivation, microbiological and Premi® tests shown respectively that 31 and 32% of samples was positive (Table 3).

Table 3. Prevalence of antibiotics residues with lysozyme inactivation.

Methods	Premi® test	Microbiological test
Positive	32 (32%)	31 (31%) [3 to 9mm]
Negative	68 (68%)	69 (69%) [< to 3mm]

n= Number of samples; ( ) = Percentage; [ ] = Minimum and maximum diameters of inhibitions zones measured.

DISCUSSION

Our results show that lysozyme, present in the sample can exert its antibacterial properties and cause false positives. So in reality, the prevalence of antibiotic residues in the meat consumed in Ouagadougou is 32%. In the case of the microbiological test the same table shows that 31% of the samples were positive. This confirms that the prevalence of antibiotic residues in meat consumed in Ouagadougou is actually 31%. When lysozyme is active we then notice a high percentage of positive whatever the used method. These results confirm the inhibitory action of lysozyme on the growth of *Geobacillus* in these samples where the meat juices did not undergo a step of lysozyme inactivation. In the case lysozyme is inactive the number of positive is relatively low regardless of the used method. So inactivation of lysozyme allows us to put aside false positives and reduce the severity of judgment that could be said about the prevalence of antibiotic residues in beef consumed in Ouagadougou.

In Burkina Faso there is a veterinary medicines registration and sale mechanism, but we observe a strong presence of street drugs in urban centers and especially in the villages. This makes the control system to be ineffective and exposes people to potential risks. Antibiotics accounted for 35% of the total volume of veterinary drugs in Burkina Faso and among these antibiotics tetracycline (oxytetracyclines) is the most used. These results corroborate with those found by Merten, (2010) where oxytetracyclines are the most used group of antibiotics in Nouakchott, Mauritania. In livestock markets, breeders ignore the concept of timeout. This is not surprising because in Burkina Faso most farmers are illiterate and 31% of those breeders practice self-medication (MRA, 2011). The works of Mitema et al. (2001) and Leopold et al. (2009) show that respectively in Kenya and Cameroon, self-medication is a common practice among breeders. Self-medication has become a habit for most Africans breeders. This practice nowadays prohibited in developed countries, could be at the origin of the selection of multidrug-resistant pathogens (Catery et al., 2003). These various results obtained from veterinary drugstores and the ones obtained from breeders attest the importance of the risk of finding antibiotic residues in beef, especially those from animals treated with antibiotics before slaughtering.

In Ouagadougou, 31% of consumed meat contains antibiotic residues. This high rate could be due to the lack of information and the practice of self-medication and the non-respect of timeout. This is worrying and must appeal the different actors of beef specialist about the poor quality of the meat in terms of chemical hazards. Comparable results were obtained in Mauritania by Merten's works in 2010. These works revealed, in fact that 58% of samples were suspects. Châtaigner and Stevens, (2005) reported that bovine meat, all races and all types of breeding combined, are heavily contaminated with antibiotic residues (about 42%). 31% positives found in Burkina Faso are below 54% positives found by Abiola et al. (2005) on antimicrobial residues in chicken liver and gizzard in the regions of "Dakar'' and "Thies'' (Senegal). This shows that Burkina Faso and all West African sub-region meat consumers are exposed to antimicrobial residues from animal source food. The identification of these residues reflects a misuse of antibiotics, essential veterinary therapeutic substances in livestock. Also uncontrolled use of antibiotics in breeding leads to the selection of resistant organisms with multiple adverse effects such as the increase of infections, mortality and decreased productivity (Klotins, 2006; Leopold et al., 2009).

Apart from these health risks, the presence of veterinary drug residues in animal source food is an important economic barrier to the international trade with must respect the new sanitary and phytosanitary rules of World Trade Organization (WTO). Lysozymes contained in the meat juice act as inhibitors to bacterial growth and may lead to false positives if they are not inactivated. These results corroborate with those found by Nouws, (2000) which stipulate that lysozymes contain bactericidal substances that can strongly inhibit the activity of bacteria in the meat juices. In Burkina Faso, as in most subregion the leaders of public health have not yet taken sufficient measures to protect consumers against chemical hazards associated with the presence of veterinary drug residues in meat. It is therefore necessary to implement a residues monitoring program.

Conclusion

The prevalence of antibiotic residues found in meat consumed in Ouagadougou in our study assumes a misuse of antibiotics by breeders and poor breeding practices in Burkina Faso. This miss use may contribute to the selection of resistant pathogens that pose a danger to public health. It should increase the awareness of policy makers on the need for strict application of antibiotic therapy in breeding. Regarding the risks associated with antibiotic residues, it is necessary to develop quality assurance programs for antibiotic residues in animal source foodstuffs. Using a chemical method such as HPLC may allow us to quantify and identify antibiotics in meat consumption in Ouagadougou.

Conflict of interests

The author(s) did not declare any conflict of interest.

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Analysis of different Namibian traditional oils against commercial sunflower and olive oils

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The current situation in many developing countries is that, vegetable oils are replacing animal fats because of health concerns and cost. The objective of the study was to compare the iodine value, acid value, ester value, peroxide value, saponification number and cholesterol content of some locally produced vegetable oils like refined marula cooking oil, marula traditional cooking oil, marula cosmetic oil, melon oil, ximenia oil viz. olive oil and commercial sunflower oil. The physicochemical analysis helps to justify the usage of these different traditional oils. The analysis showed that marula cooking oil is close to olive oil in the unsaturation level and better than olive oil in ester value, peroxide value and has lower cholesterol content.

Key words: Marula cooking oil, marula traditional oil, marula cosmetic oil, melon oil, olive oil, ximenia oil and sunflower oil.

INTRODUCTION

Food and nutrition problems are particularly severe in developing countries, many of which are located in tropical regions (Mercy et al., 2005). One of the ways of achieving food security is through the exploitation of available local resources, in order to satisfy the needs of the increasing population (Mercy et al., 2005). Lipids are one of the major constituents of foods, and are important in our diet for a number of reasons. They are a major source of energy and provide essential lipid nutrients. Nevertheless, over-consumption of certain lipid components can be detrimental to our health, for example cholesterol (not more than 200 mg/100 mL serum) and saturated fats. Vegetable oils in particular are natural lipids of plant origin consisting of ester mixtures derived from glycerol with chains of fatty acid contain about 14 to 20 carbon atoms with different degrees of unsaturation (Emmanuel and Mudiakeoghene, 2008). Vegetable oil is used as antidote to prevent some oxidative stress related diseases and a complication is advocated for different purposes (Oguntibeju et al., 2010). Vegetable oils play important functional and sensory roles in food products, and they act as carriers of fat-soluble vitamins (A, D, E and K). They also provide essential linoleic and linolenic acids, responsible for growth (Fasina et al., 2006). One

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Abbreviations: TAG, Triacylglycerol; **FA,** fatty acid; **AV,** acid value; **EV,** ester value; **SV,** saponification value.

important parameter of different vegetable oils is the amount of unsaturation of the constituent fatty acids (Nikolaos and Theophanis, 2000). It is widely known that the physical and chemical properties of oils are a strong function of the triacylglycerol (TAG) and fatty acid (FA) composition (Abdulkarim et al., 2010).

MATERIALS AND METHODS

Refined marula cooking oil, marula cosmetic oil and melon oil were supplied by Edufano Women's Cooperative oil factory, Ondangwa for the study, marula traditional cooking oil, ximenia oil were bought from an open market in Oshakati town, olive oil and sunflower oil were bought from a supermarket. Experiments in this study were done in triplicate.

Iodine value

The iodine value is expressed in grams of iodine for the amount of halogens linked with 100 g test sample, and is used as degree of unsaturated bonds of fats and oils. The higher the iodine value, the greater the degree of unsaturation. One of the most commonly used methods for determining the iodine value of lipids is "Wijs method". The lipid to be analyzed is weighed and dissolved in a suitable organic solvent, to which a known excess of iodine chloride is added. Some of the ICl reacts with the double bonds in the unsaturated lipids, while the rest remains:

$R\text{-}CH{=}CH\text{-}R + ICl_{excess} \rightarrow R\text{-}CHI\text{-}CHCl\text{-}R + ICl_{remaining}$

The amount of ICl that has reacted is determined by measuring the amount of ICl remaining after the reaction has gone to completion ($ICl_{reacted} = ICl_{excess} - ICl_{remaining}$). The amount of ICl remaining is determined by adding excess potassium iodide to the solution to liberate iodine, and then titrating with a sodium thiosulfate ($Na_2S_2O_3$) solution in the presence of starch to determine the concentration of iodine released:

$ICl_{remaining} + 2KI \rightarrow KCl + KI + I_2$

I_2 + starch + $2Na_2S_2O_3$ (blue) $\rightarrow$ 2NaI + starch + $Na_2S_4O_6$ (colourless)

The concentration of C=C in the original sample can therefore be calculated by measuring the amount of sodium thiosulfate needed to complete the titration (Determination of the iodine number of lipids, 2010). The iodine value was determined by taking 0.2 g of the sample and placing it in a 300 ml conical flask. To dissolve the sample, 10.0 ml of ethanol was added to the sample and placed in an ultrasonic bath. After dissolution, 25.0 ml Hanus solution (0.2 N ICl) added, sealed and shaken for 1 min. The solution was kept sealed and left in a dark room at about 20°C for 30 min. Ten millilitres of 15% KI and 100 ml water were added, solution sealed and shaken for 30 s. The mixture was titrated with 0.1 N $Na_2S_2O_3$ until the solution turned yellow. Five millilitre of 1% starch solution was added, solution turned blue-black colour and again titrated with 0.1 N $Na_2S_2O_3$ until the solution turned clear. The same method was performed on a blank, for the control. Calculation:

Volume of Sodium thiosulphate used = [Blank- Test] ml

$$Iodine\ No.of\ fat = \frac{Equivalent\ Wt.of\ Iodine \times Volume\ of\ Na_2S_2O_3\ used \times Normality\ of\ Na_2S_2O_3 \times 100 \times 10^{-3}}{Weight\ of\ fat\ sample\ used\ for\ analysis(g)}$$

Equivalent weight of Iodine = 127, Normality of sodium thiosulphate ($Na_2S_2O_3$) = 0.1

$$\text{Iodine value} = \frac{(B - S) \times 12.7 \times 100}{\text{Sample weight (g)} \times 1000}$$

Where, B = Blank titration, ml; S = Sample titration, ml

Saponification number

Saponification value (SV) is expressed by potassium hydroxide in mg required to saponify one (1) gram of fat. The *saponification number* is a measure of the average molecular weight of the triacylglycerols in a sample. Saponification value is inversely related to mean molecular mass. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali:

Triacylglycerol + 3 KOH $\rightarrow$ Glycerol + 3 Fatty acid salts of potassium

The lipid is first extracted and then dissolved in an ethanol solution which contains a known excess of KOH. This solution is then heated so that the reaction goes to completion. The unreacted KOH is then determined by adding an indicator and titrating the sample with HCl. The saponification number is then calculated from the knowledge of the weight of sample and the amount of KOH that reacted. The smaller the saponification numbers the larger the average molecular weight of the triacylglycerols presents (Analytical methods to measure the constants of fats and oils, 2011). The saponification value was obtained by placing 2.0 g sample in a 200 ml round bottom flask, and 25.0 ml of 0.5 M ethanolic KOH was added. The mixture was refluxed for at 65°C for 1 h. The flask was occasionally shaken while the heat was adjusted to prevent backflow of the ethanol. After heating at 65°C for 1 h, the mixture was cooled immediately and titrated with 0.5 N HCl before the test liquid solidified. A blank test was performed in triplicate to obtain the mean titre. Calculation:

$$\text{Saponification value} = \frac{(B - S) \times N \times 56.1}{\text{Sample weight (g)}}$$

Where, B = Blank titration, ml; S = Sample titration, ml; N= Normality of HCl

Acid value (AV)

The "acidity" in oil is the result of the degree of breakdown of the triacylglycerols, due to a chemical reaction called hydrolysis or lipolysis, in which free fatty acids are formed. The free fatty acidity is thus a direct measure of the quality of the oil, and reflects the care taken right from blossoming and fruit setting to the eventual sale and consumption of the oil. The acid value (AV) is defined as the mg of KOH necessary to neutralize the fatty acids present in 1 g of lipid. The acid value of oil must not be too high, as this denotes an excessively high content of free fatty acids, which causes the oil to turn sour. The lipids are extracted from the food sample and then dissolved in an ethanol solution containing an indicator. This solution is then titrated with alkali (KOH) until a pinkish colour appears. The rapid screening of acid value of fats and oils should be applied to control the quality of cooking oils. AV is considered a measure of hydrolytic rancidity. In general, it gives an indication about edibility of the lipid (Analytical methods to measure the constants of fats and oils, 2011). Five grams of fat sample were

placed in a conical flask to which 25 ml absolute ethanol was added and 3 drops of phenolphthalein. The mixture was heated in a warm bath (65°C) and occasionally shaken for 10 min after which it was cooled and titrated with 0.1 N KOH until a faint pink colour appeared. The test was done in triplicate for each sample. Calculation:

$$\text{Acid value (mg KOH/g fat)} = \frac{\text{ml KOH used for titration} \times \text{N} \times 56.1}{\text{Sample mass (g)}}$$

Where, N = Normality of KOH, % Free Fatty Acids = AV × 0.503

Ester value

Ester value (EV) is defined as the milligrams of KOH required to react with glycerine after saponification of 1 g of lipid. It is calculated from the saponification value (SV) and acid value (AV) (Analytical methods to measure the constants of fats and oils, 2011). Calculation:

EV = SV –AV

% glycerine = EV × 0.054664

Peroxide value

Peroxides (R-OOH) are primary reaction products formed in the initial stages of oxidation, and therefore give an indication of the progress of lipid oxidation. Lipid oxidation is an extremely complex process involving numerous reactions that give rise to a variety of chemical and physical changes in lipids:

Reactants → primary products → secondary products (unsaturated lipids and O_2) → (peroxides and conjugated dienes) → (ketones, aldehydes, alcohols, hydrocarbons)

One of the most commonly used methods to determine peroxide value utilizes the ability of peroxides to liberate iodine from potassium iodide. The lipid is dissolved in a suitable organic solvent and an excess of KI is added:

$ROOH + KI_{excess} \rightarrow ROH + KOH + I_2$

Once the reaction has gone to completion, the amount of ROOH that has reacted can be determined by measuring the amount of iodine formed. This is done by titration with sodium thiosulfate and a starch indicator:

I_2 + starch + $2Na_2S_2O_3$ (blue) → 2NaI + starch + $Na_2S_4O_6$ (colourless)

The amount of sodium thiosulfate required to titrate the reaction is related to the concentration of peroxides in the original sample (Determination of peroxide value, 2011). To obtain the peroxide value, 1 g of the sample was weighed and transferred to a 300 mL flask. To this 1 g of KI and 20 ml of solvent mixture (2 volumes of glacial acetic acid and 1 volume chloroform) was added and placed in a boiling water bath for 30 s. To this mixture 20 mL of 5% KI, 50 ml distilled water and 0.5 ml 1% starch solution was added. This mixture was titrated with N/500 $Na_2S_2O_3$ until solution became clear. A blank test was performed to serve as the control.

Cholesterol content

Cholesterol is a waxy steroid of fat that is produced in the liver or intestines. It is used to produce hormones and cell membranes and is transported in the blood plasma of all mammals. It is an essential structural component of mammalian cell membranes and is required to establish proper membrane permeability and fluidity. In addition, cholesterol is an important component for the manufacture of bile acids, steroid hormones, and vitamin D. Cholesterol is the principal sterol synthesized by animals. Although, cholesterol is important and necessary for mammals, high levels of cholesterol in the blood (higher than 200 mg/100 ml serum in humans) can damage arteries and are potentially linked to diseases such as those associated with the cardiovascular system (heart disease) (Okpuzar et al., 2009; Whitney and Rolfes, 2011).

Method 1: Acid ferric chloride reagent method

Standard cholesterol of 1 mg/ml was prepared in chloroform. The standard cholesterol was placed in test tubes which contained 0.1 ml of the test sample. To this 1 ml of chloroform was added. Three millilitres of acetic acid and 3 ml of acidic ferric chloride reagent were added. The mixture was left in the dark for 30 min and the absorbance was read at 560 nm. A blank test was also done which served as the control. Calculation:

Cholesterol (mg/ml) = AB/AS × CS

Where, AB = Absorbance of oil, AS = Absorbance of standard cholesterol, CS = Concentration of standard cholesterol.

Method 2: Liebermann-Buchard method

Standard cholesterol solution

Dissolve 10 mg of cholesterol in 10 ml chloroform, shake well.

Liebermann- Burchard reagent

Dissolve 0.5 mL of sulfuric acid in 10 ml of acetic anhydride. Cover and keep in ice bucket.

Sample preparation

One gram of sample was dissolved in 10 ml chloroform and further diluted to 10 times to give 10 000 ppm mixture. Three millilitres of diluted sample solution was placed in a test to which 2 ml of Liebermann-Burchard reagent and 2 ml chloroform was added. The tubes were covered with black carbon paper and kept in an ice-bucket in a dark place for 15 min. The Liebermann-Burchard reagent reacted with the sterol to produce the characteristic green colour. The absorbance was determined on a UV spectrophotometer (Helios Gamma – spectronic unicam) at 640 nm.

Working standard cholesterol solutions preparation

From the standard cholesterol solution different aliquots were pipetted (0.5, 1.0, 1.5, 2.0, 2.5 ml) into five test tubes and tube 6 was kept blank. The tubes were marked S_1, S_2, S_3, S_4, S_5 and S_6, respectively. Two millilitres Liebermann-Burchard reagent was added to all six tubes and the final volume was made up to 5 mL by adding chloroform. The tubes were covered with black carbon paper and kept in an ice-bucket in a dark place for 15 min. The Liebermann-Burchard reagent reacted with the sterol to produce the characteristic green colour. The base line was taken on the spectrophotometer with the blank (S_6) at 640 nm. The absorbance

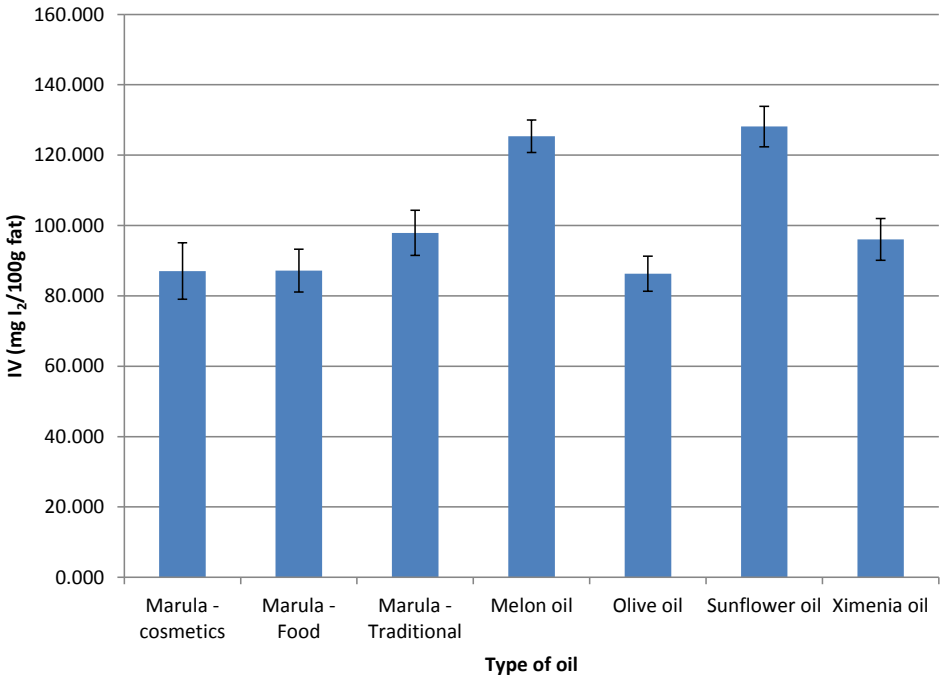

Figure 1. Iodine value of different local oils against commercial sunflower and olive oils.

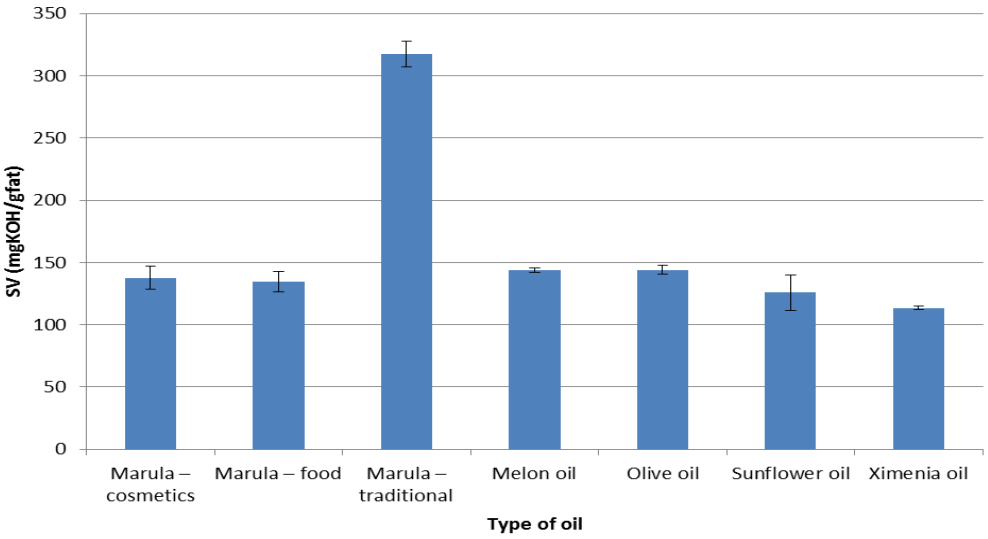

Figure 2. Saponification value of different local oils against commercial sunflower and olive oils.

of all the six tubes was determined on a UV spectrophotometer (Helios Gamma – spectronic unicam) at 640 nm and a standard graph was plotted. The absorbance of all standards (six tubes) was determined on spectrophotometer and standard graph was plotted.

RESULTS

Iodine value

In Figure 1, the highest iodine value (degree of unsaturation) was that of the sunflower oil (128.122 mg I_2/100 g oil), followed by melon oil (125.37 mg I_2/100 g oil), olive oil (86.3 mg I_2/100 g oil) and marula cooking oil was (87.2 mg I_2/100 g oil).

Saponification value

As shown in Figure 2, the highest saponification value (lowest molecular mass) was that of the marula traditional oil (317.2 mg KOH/ g oil) followed by olive oil (144 mg KOH/ g oil), marula cooking oil (134.4 mg KOH/ g oil),

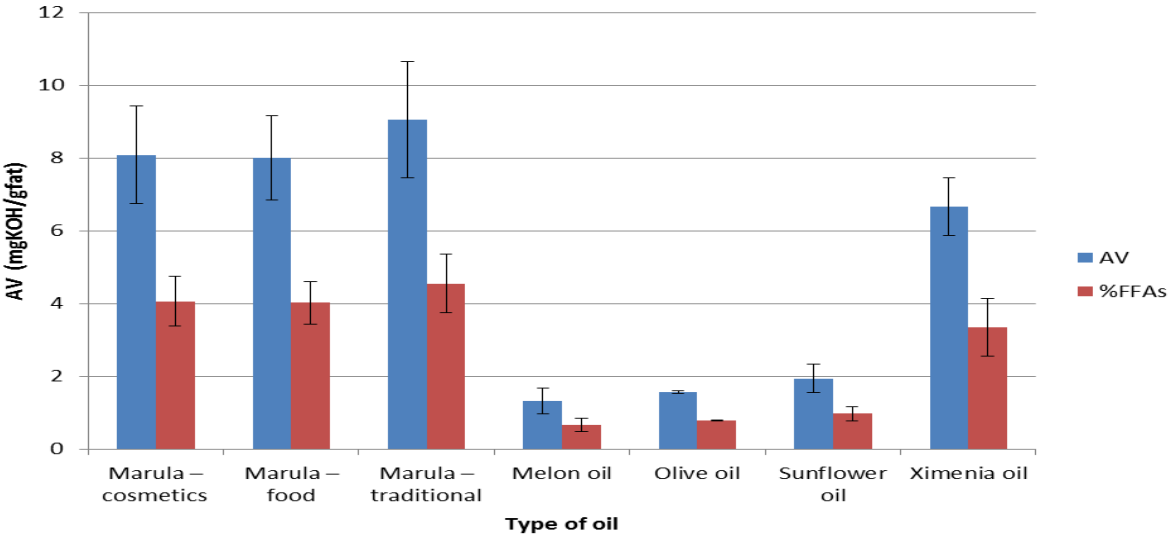

Figure 3. Acid value and free fatty acids of different local oils against commercial sunflower and olive oils.

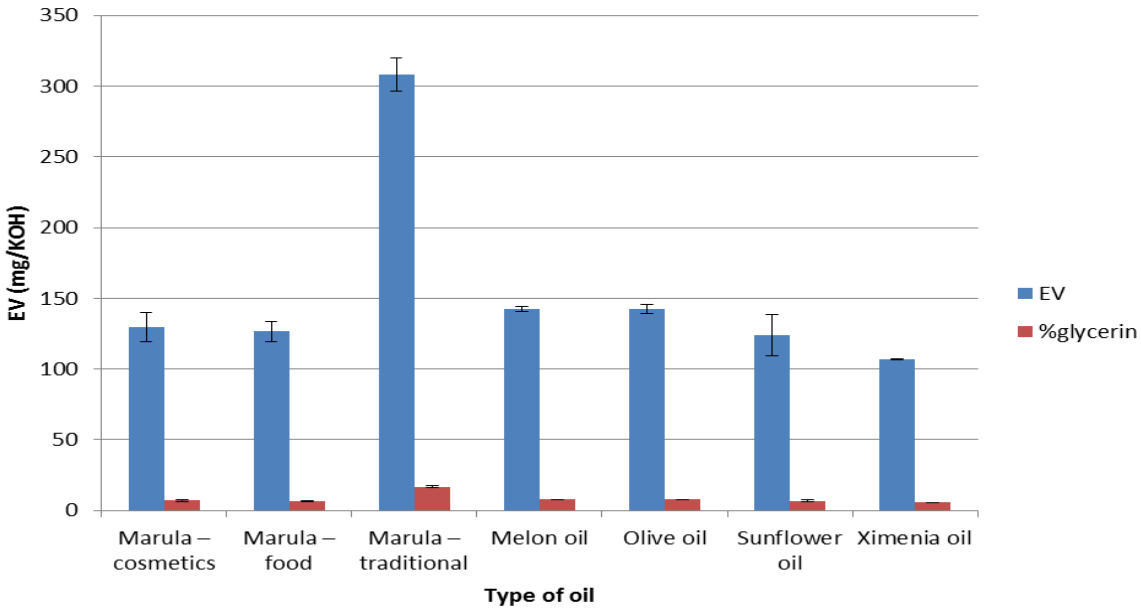

Figure 4. Ester value of different local oils against commercial sunflower and olive oils.

sunflower oil (126 mg KOH/g oil) and the lowest is the ximenia oil (113.6 mg KOH/ g oil).

Acid value and %free fatty acids

In Figure 3, the highest acid value (and free fatty acids, respectively) is marula traditional oil (9.05 mg KOH/ g of lipid), followed by marula cosmetics oil (8.08 mg KOH/ g of lipid), marula cooking oil (7.997 mg KOH/ g of lipid), sunflower oil (1.941 mg KOH/ g of lipid), olive oil (1.571 mg KOH/ g of lipid). The lowest acid values were that of melon oil (1.318 mg KOH/ g of lipid).

Ester value

In Figure 4, the highest ester value was marula traditional oil (308.16 mg KOH/ g of lipid), followed by olive oil (142.5 mg KOH/ g of lipid), melon oil (142.4 mg KOH/ g of lipid), marula cooking oil (126.44 mg KOH/ g of lipid), melon oil (142.4 mg KOH/ g of lipid), sunflower oil (124

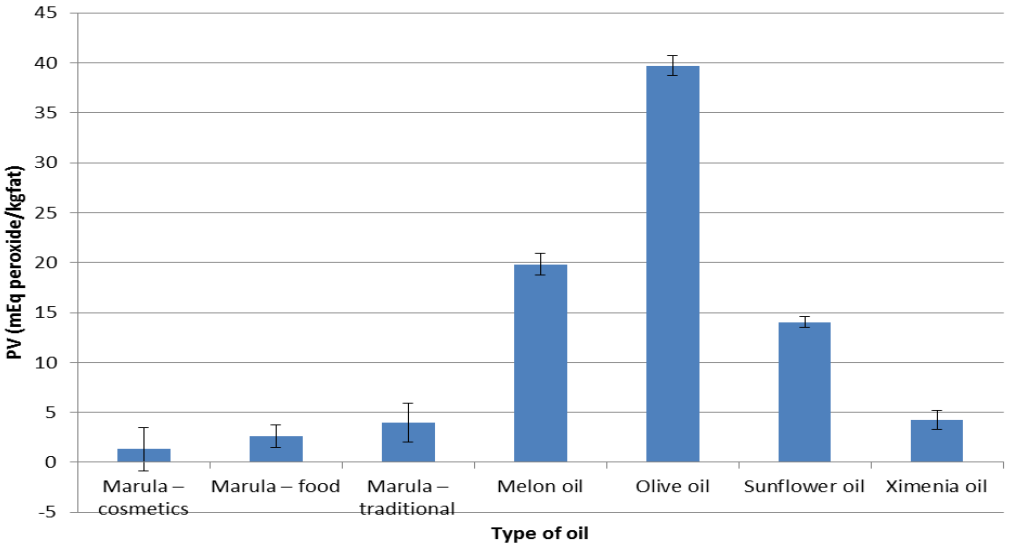

Figure 5. Peroxide value of different local oils against commercial sunflower and olive oils.

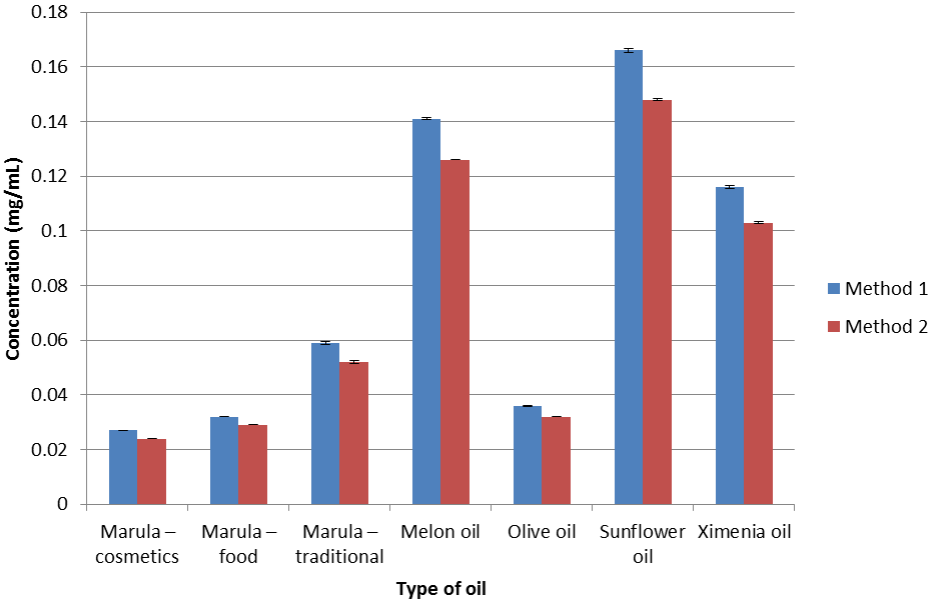

Figure 6. Cholesterol content of different local oils against commercial sunflower and olive oils.

mg KOH/ g of lipid) and the lowest was ximenia oil (106.93 mg KOH/ g of lipid).

Peroxide value

In Figure 5, the highest peroxide value was that of olive oil (39.7 mEq peroxide/Kg fat), followed by melon oil (19.8 mEq peroxide/Kg fat), sunflower oil (14.038 mEq peroxide/Kg fat), ximenia oil (4.287 mEq peroxide/Kg fat), marula cooking oil (2.657 mEq peroxide/Kg fat) and the lowest was marula cosmetics oil (1.329 mEq peroxide/Kg fat).

Cholesterol

As shown in Figure 6, the highest cholesterol content was in sunflower oil (0.166 mg/ml oil), followed by melon oil (0.141 mg/ml oil), ximenia oil (0.116 mg/ml oil), olive oil (0.036 mg/ml oil), marula cooking oil (0.032 mg/ml oil) and lowest was marula cosmetics oil (0.027 mg/ml oil).

DISCUSSION

The degree of unsaturation is measured by the iodine value. According to Whitney and Rolfes, saturated fat is considered the most detrimental to health, because it raises LDL cholesterol which leads to heart disease. The highest iodine value was that of sunflower oil (128.122 mg I_2/100 g oil), followed by melon oil (125.37 mg I_2/100 g oil), traditional marula oil (99 mg I_2/100 g oil), Ximenia oil (96 mg I_2/100 g oil), marula cooking oil was (87.2 mg I_2/100 g oil) and olive oil was the lowest (86.3 mg I_2/100 g oil). The best unsaturated traditional oil was melon oil followed by marula traditional oil and ximenia oil. The highest saponification value (lowest molecular mass) was that of the marula traditional oil (317.2 mg KOH/g oil) followed by olive oil (144 mg KOH/g oil), marula cooking oil (134.4 mg KOH/g oil), sunflower oil (126 mg KOH/g oil) and the lowest is the ximenia oil (113.6 mg KOH/g oil). The average molecular weight of the triacylglycerols in marula traditional oil is lowest and ximenia oil is the highest. The acid value must not be too high; because it is a result of the breakdown of triacylglycerols. High acid value causes the oil to turn sour. The highest acid value (and free fatty acids, respectively) is marula traditional oil (9.05 mg KOH/g of lipid), followed by marula cosmetics oil (8.08 mg KOH/g of lipid), marula cooking oil (7.997 mg KOH/g of lipid), sunflower oil (1.941 mg KOH/ g of lipid), olive oil (1.571 mg KOH/g of lipid). The lowest acid values were that of melon oil (1.318 mg KOH/g of lipid). It should be noted that the marula oils were kept in the laboratory for sometime before the analysis, which may have led to increasing the acid value. The highest ester value was marula traditional oil (308.16 mg KOH/ g of lipid), followed by olive oil (142.5 mg KOH/ g of lipid), melon oil (142.4 mg KOH/g of lipid), marula cooking oil (126.44 mg KOH/ g of lipid), sunflower oil (124 mg KOH/ g of lipid) and the lowest was ximenia oil (106.93 mg KOH/ g of lipid). The ester value of ximenia oil is the lowest among traditional oils followed by marula cooking oil and melon oil.

Foods which contain high concentrations of unsaturated lipids are particularly susceptible to lipid oxidation. Lipid oxidation is one of the major forms of spoilage in foods, because it leads to the formation of off-flavours and toxic compounds and is measured by the peroxide value (Analytical methods to measure the constants of fats and oils, 2011). The highest peroxide value was that of olive oil (39.7 mEq peroxide/kg fat), followed by melon oil (19.8 mEq peroxide/kg fat), sunflower oil (14.038 mEq peroxide/kg fat), ximenia oil (4.287 mEq peroxide/kg fat), marula traditional oil (3.8 mEq peroxide/kg fat), marula cooking oil (2.657 mEq peroxide/kg fat) and the lowest was marula cosmetics oil (1.329 mEq peroxide/kg fat). The peroxide value of marula cosmetics oil is the lowest followed by marula cooking oil and then ximenia oil. High levels of cholesterol in blood (more than 200 mg/100 ml serum) can damage arteries and are potentially linked to diseases such as those associated with the cardiovascular system (heart diseases). Highest cholesterol content was in sunflower oil (0.166 mg/ml oil), followed by melon oil (0.141 mg/ml oil), ximenia oil (0.116 mg/ml oil), marula traditional oil (0.057 mg/ml oil), olive oil (0.036 mg/ml oil), marula cooking oil (0.032 mg/ml oil) and lowest was marula cosmetics oil (0.027 mg/ml oil). The cholesterol level of marula cosmetics oil has the lowest cholesterol content followed by marula cooking oil and then marula traditional oil.

Conclusion

When looking at the results of different tests above, it is found that melon oil has the highest iodine value (highest unsaturation value), marula traditional oil has the lowest molar mass of the triacylglycerols, melon oil has the lowest acid value, ximenia oil has the lowest ester value, marula cosmetics has the lowest peroxide value and the lowest cholesterol content. It is very hard to say which is the overall best oil, however, marula (refined) cooking oil has low cholesterol content, relatively low acid value, ester value, peroxide value and reasonable saturation. It is close to olive oil in unsaturation level and better than olive oil in ester value, peroxide value and cholesterol level. The physicochemical data obtained, confirm that the traditional oils (edible and inedible) have characteristics that are comparable to their commercial counterparts. This indicates that these traditional oils can be used for cooking purposes. However, further studies to evaluate their toxicity should be conducted next.

Conflict of interests

The author(s) did not declare any conflict of interest.

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Sensory profile of fermented milk drinks flavored with fruits from the Brazilian Cerrado

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The Brazilian cerrado has several fruit species with great potential for agro-industrial use including the production of milk drinks. Thus, the objective of this study was to evaluate the sensory profile and purchase intent of fermented milk drinks added in increasing levels of fruits from the Brazilian cerrado. Four formulations of milk drinks were processed with concentrations of 4, 8, 12 and 16% of Araçá (*Psidium cattleianum*), Araticum (*Annona crassiflora* Mart), Mangaba (*Hancornia speciosa Gomes*), Passion fruit (*Passiflora edulis* f. flavicarpa) and Pequi pulps (*Caryocar brasiliense* Camb.). The sensory profile of products was characterized by affective test evaluating acceptance using the hedonic scale and purchase intent. Fruits used in flavoring, especially Pequi, are very appreciated by the local population. As demonstrated in acceptability tests, it is believed that this factor contributed to the high acceptance of Pequi. All milk drinks showed positive purchase intent value.

Key words: Fermented milk, acceptance test, purchase intent.

INTRODUCTION

Fermented milk originates from fermentation with production of lactic acid as a final product. Acidification is responsible for extending the shelf-life of food products (Finco, 2011). According to Oliveira et al. (2013), economic stability and increased consumer demand are responsible for the increased production of milk drinks on an industrial scale in Brazil.

Brazil has several fruit species with great potential for agro-industrial use, many of them are used by the population and processed into juices, liqueurs, jams and candies or even through fresh consumption (Silva et al., 2008).

The functional properties of fruits are responsible for their increased consumption, mainly due to the presence of bioactive substances that have positive physiological effects through the antioxidant action even with inexpressive consumption (Melo et al., 2008). Many fruits from the Brazilian cerrado are included in the human diet; however, many fruit species with food, agro-industrial and economic potential need to be researched to definitely be

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Table 1. Formulations of fermented milk drinks containing Araçá, Araticum, Mangaba, Passionfruit and Pequi pulp.

Ingredients (1000 g mass)	Fruit pulp proportion (%)			
	4	8	12	16
Milk (g)	500	500	500	500
Milk whey (g)	200	200	200	200
Sucrose (g)	100	100	100	100
Milk powder (g)	30	30	30	30
Pulp (g)	40	80	120	160
Water (pH and TSS = pulp)	130	90	50	10
Probiotic lactic yeast	400 mg	400 mg	400 mg	400 mg

part of the regular diet and the formal fruit market.

Fermented milk drinks were flavored with Araçá, Araticum, Mangaba, Passion fruit and Pequi constitute products with high palatability and nutritional value due to the use of natural raw materials and without use of preservatives.

The Cerrado is the second largest biome in South America, from the point of view of biological diversity; the Brazilian Cerrado is recognized as a savanna harboring many species of native plants that have already been cataloged. In this context, the aim of this study was to evaluate the sensory profile and purchase intent of fermented milk drinks added with increasing levels of fruit pulp from the Brazilian cerrado.

MATERIALS AND METHODS

Fruit pulp from the Brazilian cerrado

Fruits were collected directly from Araca, Araticum, Mangaba, Passion fruit and Pequi plants in the Cerrado region, southwestern state of Goiás. Fruits were selected, sanitized with 100 ppm sodium hypochlorite / 10 min.

The fruits of Araca and Pequi were manually pulped, the outer shell removed and the pulp was withdrew with a knife. The fruits of Araticum, Mangaba and passion fruit were peeled manually and were later taken to industrial removing device coupled with stainless steel sieve separating the pulp from the seeds. The fruit pulps were packed in polyethylene bags, identified and frozen for later use.

Fermented milk drinks

To prepare the milk base, 40% milk whey and 60% milk (mass / mass) were used. Also, 10% sucrose and 3% milk powder were added to the milk base with heating at 90°C / 3 min followed by cooling to 42°C. *Lactobacillus acidophilus* (La-5), Bifidobacterium (BB-12) and *Streptococcus thermophilus* cultures for fermentation were used in the amounts specified by the manufacturer.

The fermentation of the milk drink occurred in an oven at 42°C until it reached pH 4.5. Later, the mass was cooled to 20°C for homogenization and fruit pulps were added.

To be added to fermented milk drinks, pulps were pasteurized at 70°C for 3 min. The content of total soluble solids (TSS) was obtained with the use of bench top refractometer. Pulps were used in the proportions of 8, 12 and 14 and 16% in relation to the milk drink mass.

Aiming to make the pulp more homogeneous when added to the milk base, special water was prepared, with pH and TSS identical to the pulp to be added. The water pH was adjusted with the addition of ascorbic acid and sucrose to reach the desired TSS.

Araçá, araticum, mangaba, passion fruit and pequi pulps were diluted in the respective waters with pH and TSS equal to the respective pulps and added to the fermented milk base corresponding to each experiment. Then, milk drinks were packaged in 200-mL polyethylene packages to perform the sensory analysis.

Five trials according to the different fruit pulps were performed and distributed into four treatments as follows: Treatment 1 - milk drink added with 4% pulp; Treatment 2 - milk drink added with 8% pulp; Treatment 3 - milk drink added with 12% pulp and Treatment 4 - milk drink added with 16% pulp. Each treatment had three replicates according to Table 1.

Microbiological analyses

The detection and enumeration required to establish the total count of yeasts and molds whose unit is given in colonies forming units (CFU) was performed using method for dairy products through the technique of colony count at 25°C described by ISO 6611: IDF 94 (2004).

The most probable number of total coliforms was established in accordance with ISO 4831 (2006).

Sensory analysis

Sensory characteristics were assessed in order to quantify the preference of consumers for milk drinks flavored with different contractions concentrations of cerrado fruits such as Araçá, Araticum, Mangaba, Passionfruit and Pequi, as well as their purchase intent.

On the eighth day after processing, sensory analyses of milk drinks flavored with Cerrado fruits were performed.

Sensory evaluation was performed using affective method by Acceptance Testing with a 9-point Hedonic scale, where one (1) represented "disliked very much"; two (2) "dislike much"; three (3) "disliked regularly"; four (4) "disliked slightly"; five (5) "neither liked nor disliked"; six (6) "liked slightly"; seven (7) "liked regularly"; eight (8) "liked much" and nine (9) "liked very much" in order to judge the sensory attributes according to parameters of color, aroma, flavor, acidity, viscosity and appearance, also evaluating the purchase intent of consumers (IAL , 2005). 50 untrained panelists were used. Sensory evaluations were performed at the Laboratory for Sensory Analysis of the Food Engineering Course, IF Goiano, Rio Verde

Table 2. Comparison of the mean values and concordance coefficient among judges (CC) regarding sensory parameters of color, aroma, flavor, acidity, viscosity and appearance of milk drink with increasing levels of Araçá pulp (*Psidiumcattleianum*).

Sensory parameters	Araçá Pulp (%)			
	4	8	12	16
Color	6.40a	6.30a	6.32a	6.53a
CC (%)	24.88	29.74	32.59	25.85
Aroma	6.57a	6.43a	6.53a	6.74a
CC (%)	32.74	28.86	36.6	35.08
Flavor	6.11a	5.36a	5.38a	5.66a
CC (%)	22.96	16.82	19.12	16.52
Acidity	6.51a	5.98a	5.81a	5.98a
CC (%)	27.68	27.68	26.66	23.84
Viscosity	5.74a	5.62a	5.60a	5.96a
CC (%)	21.12	26.08	21.59	22.4
Appearance	6.00a	5.64a	5.77a	5.83a
CC (%)	18.87	23.05	26.85	22.96

Different letters in the line are significantly different by the Tukey test at 5% probability.

Campus, conducted in individual booths under white light. Samples labeled with three digits consisting of 20 ml of milk drink from each treatment randomly distributed were provided to tasters who consented to participate in the study along with the form to be filled.

In the sensory evaluation form, it was possible to provide both sensory and purchase intent parameters and additional information such as age, gender, affinity and frequency of the consumption of milk drinks.

Statistical analysis

The results of the sensory profile and purchase intent of milk drinks were evaluated according to each experiment in a completely randomized design.

Means of sensory evaluation were compared by the Tukey test at 5% probability using the SISVAR 5.3 software (Ferreira, 2010).

The concordance coefficient (CC) among judges determines the percentage of tasters who agree with each mean obtained by the evaluation of sensory attributes based on the hedonic scale. CC was obtained through the CONSENSOR 1.1 software (Silva et al., 2010).

The Acceptability Index (AI) was obtained using the formula: AI (%) = A x 100 / B, where A is the value of the mean score obtained for the product and B is the highest score given to the product. For the milk drink to have positive acceptance as for the sensory attributes, the AI value should be equal to or higher than 70% (Teixeira et al., 1987).

RESULTS AND DISCUSSION

The results of the total count of yeasts and molds for all milk drinks obtained was score <1.0x10¹ CFU, which is within limits established by law andaccording to ISO 6611: IDF 94 (2004). The mean result of the most probable number for Total Coliforms was less than 0.30 MPN / mL, which is within the limit established by ISO 4831 (2006).

Fifty untrained panelists aged 17-52 years participated in the evaluation of the sensory characteristics of fermented milk drinks; 44% of participants were male and 56% female.

When assessing the affinity of tasters in relation to the consumption of dairy drinks, 98% of participants reported liking milk drinks and only 2% reported otherwise.

Regarding the frequency of the consumption of dairy drinks, 26% of tasters reported consuming every day, 37% consume dairy drinks once a week, 2% reported consuming every two weeks, and 35% once a month. Thus, it was observed that the consumption of fermented dairy drinks regardless of flavor is quite significant, justifying the development of dairy drinks with different flavors, attracting the interest of consumers to the variation of flavors, especially typical and characteristic flavors of the cerrado region.

Tables 2 to 6 show the mean sensory profile characterization values and the concordance coefficient (CC) among judges determined in the four treatments of fermented dairy drinks with increasing levels of Araçá (*Psidium cattleianum*) Araticum (*Annona crassiflora Mart*), Mangaba (*Hancorniaspeciosa Gomes*), Passion fruit (*Passifloraedulis f. flavicarpa*) and Pequi pulp (*Caryocarbrasiliense Camb*.) after eight days of storage, because after this time the milk drink has better flavor and texture, acquired the appropriate characteristics according to Oliveira and Damin (2003) report that at this time there is greater viability of lactic acid bacteria.

In Table 2, when comparing the mean values by the Tukey test, all sensory parameters of all treatments with

Table 3. Means and concordance coefficient (CC) among judges for the sensory analysis of milk drinks with increasing levels of Araticum pulp (*Annona crassiflora Mart*) for parameters of color, aroma, flavor, acidity, viscosity and appearance.

Sensory parameters	Araticum Pulp (%)			
	4	8	12	16
Color	6.50a	6.30a	6.39a	5.48a
CC (%)	25.84	25.61	27.79	20.68
Aroma	6.36a	6.25a	6.02a	5.75a
CC (%)	24.61	21.47	23.48	18.81
Flavor	6.80a	5.93a	5.86a	5.73a
CC (%)	29.02	23.48	16.51	16.5
Acidity	6.39a	5.86a	6.14a	5.64a
CC (%)	21.68	19.73	23.69	15.53
Viscosity	6.30a	5.84a	5.98a	5.50a
CC (%)	17.86	22.98	16.51	14.24
Appearance	6.43a	6.07a	6.07a	5.91a
CC (%)	24.16	26.79	24.92	27.61

Different letters in the line are significantly different by the Tukey test at 5% probability.

Table 4. Means and concordance coefficient among judges (CC) of the sensory analysis of milk drink with increasing levels of Mangaba pulp (*Hancorniaspeciosa Gomes*) for parameters of color, aroma, flavor, acidity, viscosity and appearance.

Sensory parameters	Mangaba pulp (%)			
	4	8	12	16
Color	6.28a	6.38[a]	6.30a	6.40a
CC (%)	21.07	28.72	27.35	31.74
Aroma	5.96a	5.70a	5.78a	5.86a
CC (%)	17.28	21.02	22.07	20.66
Flavor	5.76a	5.02a	5.10a	4.92a
CC (%)	17.78	14.42	12.77	18.59
Acidity	6.16a	5.56a	5.74a	5.30a
CC (%)	24.48	24.39	23.26	15.33
Viscosity	5.92a	5.84a	5.74a	5.68a
CC (%)	22.27	25.34	18.03	20.15
Appearance	6.28a	6.10a	5.96a	5.94a
CC (%)	25.85	23.06	21.45	14.91

Different letters in the line are significantly different by the Tukey test at 5% probability.

increasing addition of Araçá pulp showed no significant difference ($p < 0.05$), that is the Araçá pulp concentration did not significantly influence the evaluation of tasters.

Milk drink added with 12% Araçá pulp presented mean parameters of color, aroma and appearance with the highest concordance coefficient (CC) among judges.

Therefore, flavor and acidity of milk drink added of 4% Araçá pulp showed the highest concordance coefficient among judges (CC) compared to the means obtained, demonstrating greater preference of consumers for milk drink with lower pulp concentrations.

For parameter of viscosity, milk drink with 8% Araçá pulp showed the highest concordance coefficient among judges with the mean of 5.62.

For parameters of color and aroma, all treatments of milk drink flavored with Araçá were acceptable. For parameter

Table 5. Means and concordance coefficients among judges (CC) of the sensory analysis of milk drink with increasing levels of Passion fruit pulp (*Passifloraedulis f. Flavicarpa*) for parameters of color, aroma, flavor, acidity, viscosity and appearance.

Sensory Parameters	Passion fruit pulp (%)			
	4	8	12	16
Color	6.38b	6.80ab	7.09ab	7.31a
CC (%)	30	34.12	32.32	36.51
Aroma	7.11a	7.20a	7.13a	7.33a
CC (%)	33.67	31.8	31.97	35.59
Flavor	6.89a	6.91a	6.24a	6.20a
CC (%)	32.49	29.63	21.61	23.09
Acidity	6.49a	6.24ab	5.93ab	5.24b
CC (%)	29.63	22.85	19.82	15.99
Viscosity	5.29a	5.96a	5.89a	6.22a
CC (%)	27.87	24.72	22.85	26.03
Appearance	5.78b	6.47ab	6.60ab	7.00a
CC (%)	24.72	31.15	30.18	33.5

Different letters in the line are significantly different by the Tukey test at 5% probability.

Table 6. Means and concordance coefficients among judges (CC) of the sensory analysis of milk drinks with increasing levels of Pequi pulp (*Caryocarbrasiliense Camb.*) for parameters of color, aroma, flavor, acidity, viscosity and appearance.

Sensory Parameters	Pequi Pulp (%)			
	4	8	12	16
Color	7.05a	7.38a	7.67a	7.76a
CC (%)	33.5	44.61	45.74	50
Aroma	6.52a	7.10a	6.81a	6.71a
CC (%)	36.11	40.41	39.77	25.73
Flavor	7.14a	7.33a	7.33a	6.76a
CC (%)	39.77	42.86	41.03	36.42
Acidity	6.29a	6.95a	6.76a	6.62a
CC (%)	27.66	36.8	33.5	30.14
Viscosity	6.95a	7.00a	6.95a	7.24a
CC (%)	32.73	44.18	34.99	36.42
Appearance	6.67ª	7.10a	6.90a	7.38a
CC (%)	29.45	44.03	33.5	45.18

Different letters in the line are significantly different by the Tukey test at 5% probability.

of acidity, only milk drink with 4% Araçá pulp obtained the higher percentage of acceptability and for the other parameters and treatments, products did not obtain satisfactory acceptability.

Therefore, in relation to parameter of taste, milk drink added of Araçá pulp was not well accepted by tasters, which could be due to the fact that Araçá pulp has many seeds yielding milk drink with residues, which may not have pleased judges satisfactorily.

Table 3 shows the mean values for the sensory profile characterization and the concordance coefficient among judges (CC) determined in the four treatments of fermented milk drink with increasing levels of Araticum pulp (*Annona crassiflora Mart*) after eight days are storage.

Assessing all sensory parameters of milk drinks with increasing levels of Araticum pulp and comparing by the Tukey test, no significant difference ($p > 0.05$) among treatments was found.

When evaluating parameters of color and acidity, the

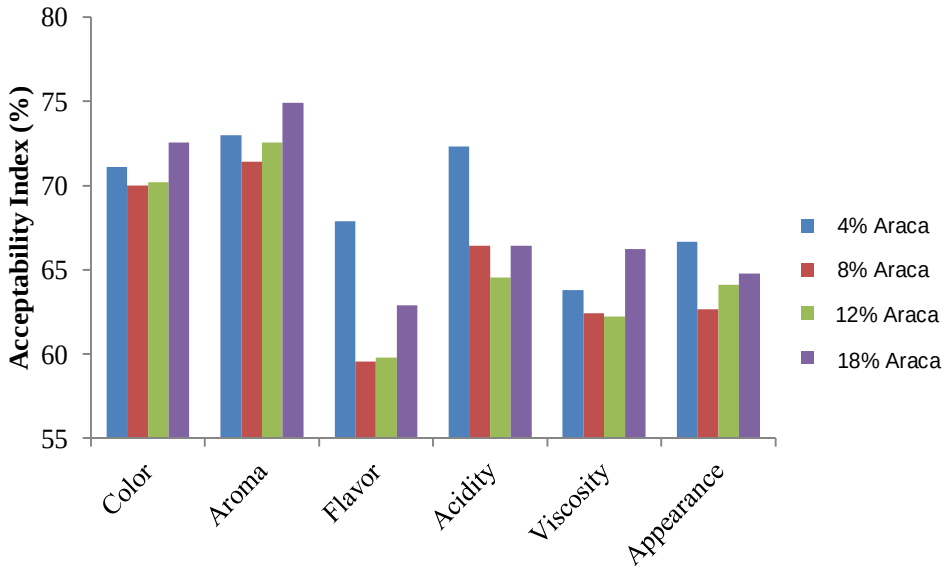

Figure 1. Acceptability Index of the sensory attributes of dairy drink with increasing levels of Araca pulp.

concordance coefficient among judges was higher for milk drink added with 12% of Araticum pulp.

Parameters of aroma and flavor of milk drink with the lowest level of Araticum pulp (4%) showed the highest concordance coefficient among judges, and it could be inferred that even the lowest fruit concentration provided the product characteristic aroma and flavor.

When evaluating parameter of viscosity, concordance coefficient among judges was higher for milk drink added with 8% Araticum pulp (5,84); and for parameter of appearance, judges agree that the mean scores assigned is close to 5.91 for the addition of 16% Araticum pulp. These values can be explained because Araticum pulp has sandy texture, as well as the product added of it, and increasing the addition of Araticum pulp proportionally increases the sandy texture of the final product, which may not have pleased judges satisfactorily.

When the concordance coefficient among judges is around good mean or high mean, the acceptability index of that parameter is above 70%, which can be seen in Figure 1, showing the acceptability index of the sensory profile of milk drinks flavored with increasing levels of Araticum pulp.

When assessing parameter of color, milk drink added with Araticum pulp showed acceptability for treatments with 4, 8 and 12% Araticum pulp. For parameter of aroma, treatments with 4 and 8% were acceptable, and in relation to parameters of flavor, acidity, viscosity and appearance, only treatment with the addition of 4% Araticum pulp was accepted.

Rocha et al. (2008) evaluated the addition of Araticum jam to fermented milk drink and obtained good acceptance for the product, similar to the addition of 4% Araticum pulp in the present study.

Figure 2 shows the sensory acceptability index of milk drink with increasing levels of Araticum pulp.

It could be inferred that although intermediate levels of pulp intensify attributes of color and aroma, minimum level of Araticum pulp was enough to please tasters.

Table 4 shows that the values of sensory parameters showed no significant difference ($p < 0.05$) from each other, that is, the Araçá pulp concentration did not influence the assessment of tasters.

The concordance coefficient among judges was higher for parameters of color and flavor of milk drink added with 16% Mangaba pulp. Color showed the highest mean (6.40), and flavor showed the lowest mean (4.92), and it could be inferred that high amounts of Mangaba pulp enhance the color of the product; however, the product obtains strong or cloying flavor, making judges to be indifferent or dislike it slightly.

Regarding aroma, the concordance coefficient was higher for milk drink added with 12% Mangaba pulp, and for parameter viscosity, the concordance coefficient was higher for milk drink added with 8% Mangaba pulp, and intermediate values of Mangaba pulp addition expressed means that indicate indifference of tasters in relation to these factors.

For milk drink added with 4% Mangabap ulp, parameters of acidity and appearance obtained the highest means, followed by higher concordance coefficient among judges.

Means and concordance coefficients for milk drink added with Mangaba pulp were similar to means found by Silva (2013), assessing yogurts added with Umbu pulp, and for attributes of appearance, aroma, flavor, means obtained for yogurt correspond in scale to "neither liked nor disliked" identifying indifference of judges regarding the acceptance of yogurts.

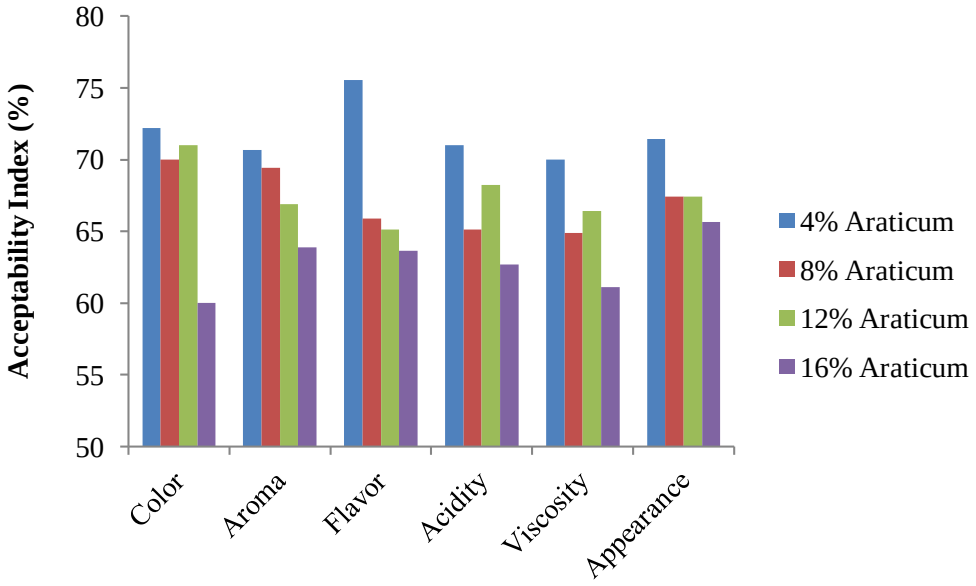

Figure 2. Acceptability index of the sensory attributes of milk drink with increasing levels of Araticum pulp.

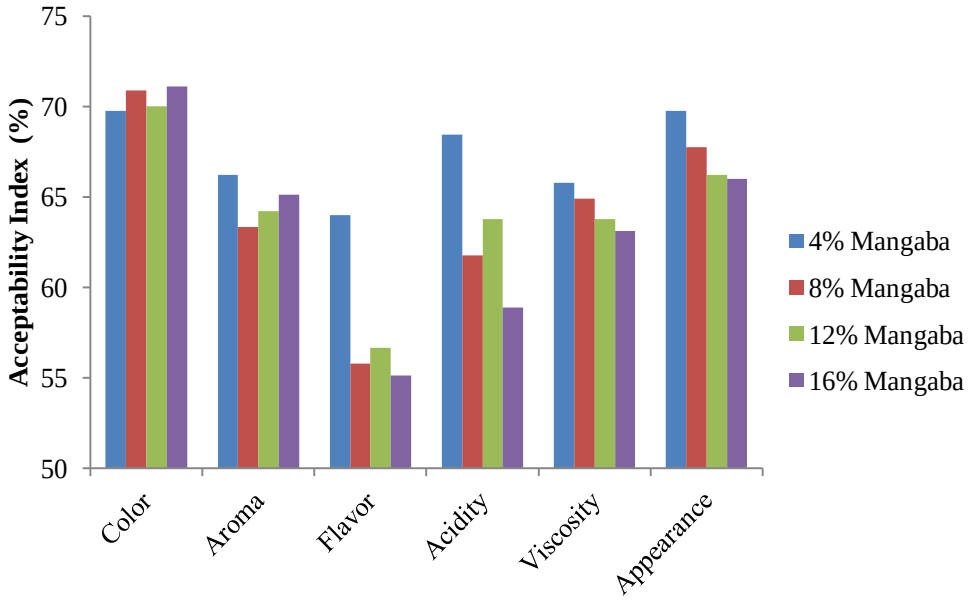

Figure 3. Acceptability index of the sensory attributes of milk drink with increasing levels of Mangaba pulp.

Figure 3 shows the sensory acceptability index of milk drink with increasing levels of Mangaba pulp.

In milk drink flavored with Mangaba, only treatments with the addition of 8, 12 and 16% of Mangaba pulp obtained good acceptance for parameter of color. The other parameters and treatments did not obtain acceptance values higher than 70%.

The study by Rocha et al. (2008) evaluating the addition of Mangaba jamto fermented milk drink, showed acceptability similar to milk drink added with 4% Mangaba pulp. Thus, milk drink added with Mangabapulp was better accepted in minimal levels (4%).

In Table 5, when evaluating parameters of color and appearance, means showed no significant difference

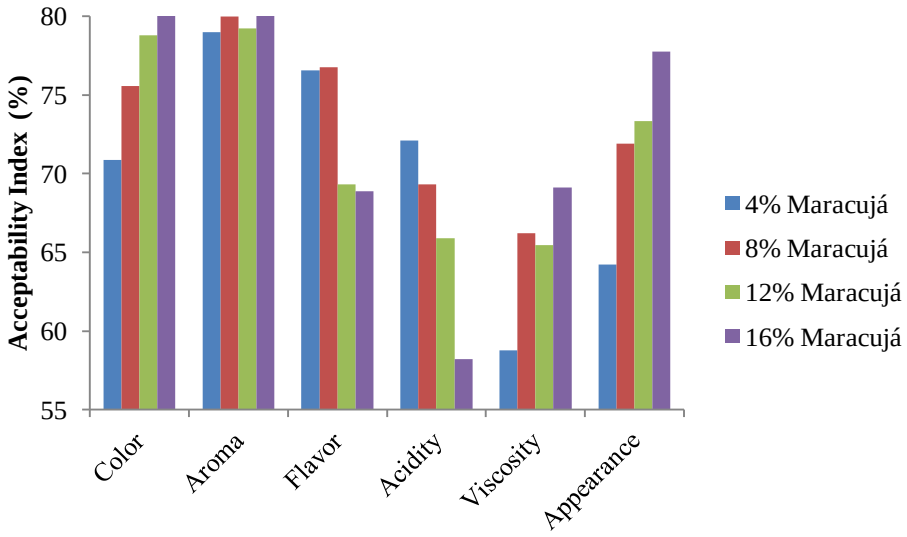

Figure 4. Acceptance index of the sensory attributes of milk drink with increasing levels of passion fruit pulp.

($p<0.05$) among treatments with the addition of 8 and 12% passion fruit pulp, and only treatment with the addition of 4% passion fruit pulp showed significant difference ($p <0.05$).

For parameter of aroma, no significant difference ($p <0.05$) between means was found. Therefore, the addition of high amounts of passion fruit pulp enhanced color and flavor of the product, thereby positively affecting appearance.

Means for parameters flavor and viscosity of milk drink with the lowest addition of passion fruit pulp (4%) showed the highest concordance coefficients among judges, with no significant difference ($p <0.05$) for the means of these parameters.

When evaluating parameter of acidity, milk drink added with 4% passion fruit pulp showed the highest concordance coefficients among judges for the highest mean (6.49), differing ($p <0.05$) from each other.

When evaluating parameter of acidity, milk drink added with 4% passion fruit pulp showed the highest concordance coefficients among judges for the highest mean (6.49), and no difference ($p <0.05$) was significant of samples with addition of 8 and 12% pulp.

The means of treatments with 8 and 12% passion fruit pulp showed no significant difference ($p <0.05$) from each other.

The highest concordance coefficients regarding parameters of color, aroma and appearance are given for the highest means in milk drink added with 16% passion fruit pulp.

Figure 4 shows the sensory acceptance index of milk drink with increasing levels of passion fruit pulp.

In relation to parameters of color and aroma for milk drink added with passion fruit pulp, all treatments were well accepted. For parameter of flavor, treatments with 4 and 8% of passion fruit pulp obtained good acceptability.

Appearance was well accepted in treatments with 8, 12 and 16% passion fruit pulp, and viscosity showed no acceptability in any treatment.

Almeida et al. (2013) assessed the production and sensory characterization of yogurt enriched with passion fruit pulp and reported that samples added of 10 and 12% passion fruit had greater acceptance, and the addition of passion fruit pulp provides flavor with desirable acidity and acceptable by judges.

In Table 6, when comparing means by the Tukey test, all sensory parameters and all treatments with increasing levels of Pequi pulp showed no significant difference ($p <0.05$) from each other.

Milk drink added with 16% Pequi pulp showed the highest concordance coefficients among judges for the highest means, respectively (7.76 and 7.38) and when evaluating parameters of color and appearance, a higher Pequi pulp concentration enhanced the color of products and as appearance is directly linked to visual, it means that tasters liked the product.

For milk drink added with 8% Pequi pulp for parameters of aroma, flavor and acidity, the highest means followed by the highest concordance coefficients among judges were obtained.

For milk drink added with 8% Pequi pulp for parameters of aroma, flavor and acidity, there was no significant difference.

One of the most important parameters in the sensory evaluation is flavor, which stands out both for scores corresponding to liked moderately as for concordance coefficients exceeding 40% (Marinho et al., 2012).

For parameter of viscosity, milk drink added with 8% Pequi pulp obtained the highest concordance coefficients

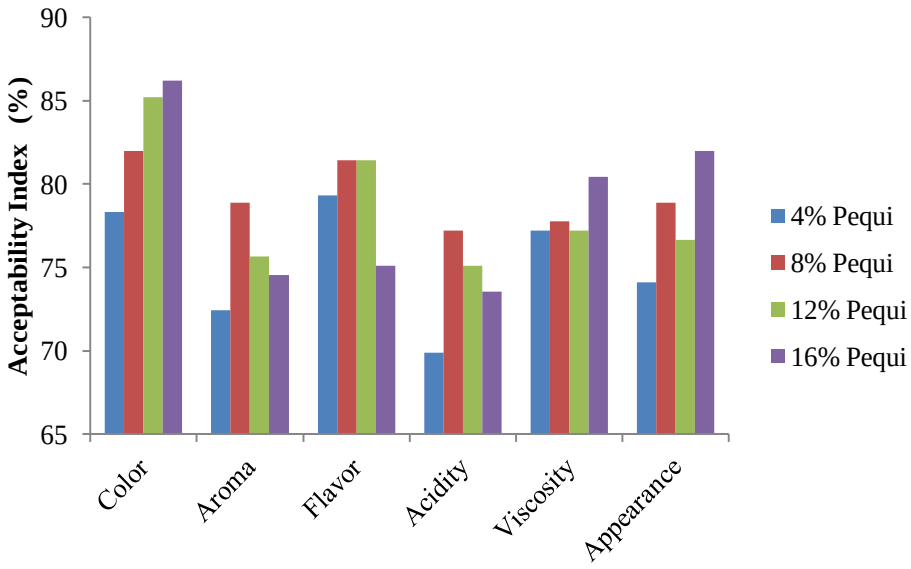

Figure 5. Acceptability Index of the sensory attributes of milk drink with increasing levels of Pequi pulp.

among judges. This result shows that when it comes to a fruit with very strong and distinctive flavor as Pequi, intermediate values of pulp addition are sufficient to characterize the milk drink as expected because the means for the addition of 8% Pequi pulp indicate that the judges liked the product. This result shows that intermediate values of pulp addition are sufficient to characterize the milk drink according to expected because the means indicate that the judges liked the product

Figure 5 shows the sensory acceptabilityindex of milk drink with increasing levels of Pequi pulp.

With respect to the addition of Pequi pulp, milk drink and its acceptability, all treatments in all parameters had excellent acceptability index, except fortreatment with the addition of 4% Pequi pulp for parameter of acidity, indicating that tasters approved the addition of Pequi pulp to the milk drink.

Assessment of the acceptability index shows that intermediate additions of pulp (8 and 12%) obtained good results for parameters of aroma, flavor and acidity.

It could be inferred that because fruits used in the study are typical of the Brazilian cerrado where the research took place, tasters would have greater affinity with them. The study by Brasil et al. (2011) showed similar acceptability, where milk drink added with 8% Pequi pulp was well accepted by judges.

Fermented milk drinks flavored with Araçá, Araticum, Mangaba, Passion fruit and Pequi constitute products with high palatability and without use of preservatives.

Figure 6 shows the comparison on the purchase intent related to each addition of pulp to milk drinks.

The Purchase Intent was assessed with the following question to tasters "Would you buy milk drink flavored with Araçá?", and the same question was made to tasters who did the sensory analysis in relation to the other fruits.

In general, it could be inferred that the purchase test obtained positive result, since all the drinks showed value exceeding 50% for option "Yes" (yes, I would buy the product).

Milk drink flavored with passion fruit pulp had the highest percentage of Purchase Intent (98%), followed by milk drink flavored with Pequi pulp (77%). This is because passion former is widely known and common, and Pequi stands for being a very popular fruit in the region.

Milk drink flavored with passion fruit pulp had the highest percentage of Purchase Intent (98%), followed by milk drink flavored with Pequi pulp (77%). This is because varieties passion fruit are known, and Pequi stands for being a very popular fruit in the region.

Conclusion

The fruits used to flavor milk drinks, especially Pequi, are much appreciated by the local people. As demonstrated in acceptability tests, it is believed that this factor contributed to the prominence acceptance of Pequi. All milk drinks showed positive Purchase Intent values.

The economic viability of products is clear, since it is of easy preparation and has low production cost due to the use of milk whey. The potential for economic exploitation of native Cerrado species is high.

Conflict of interests

The author(s) did not declare any conflict of interest.

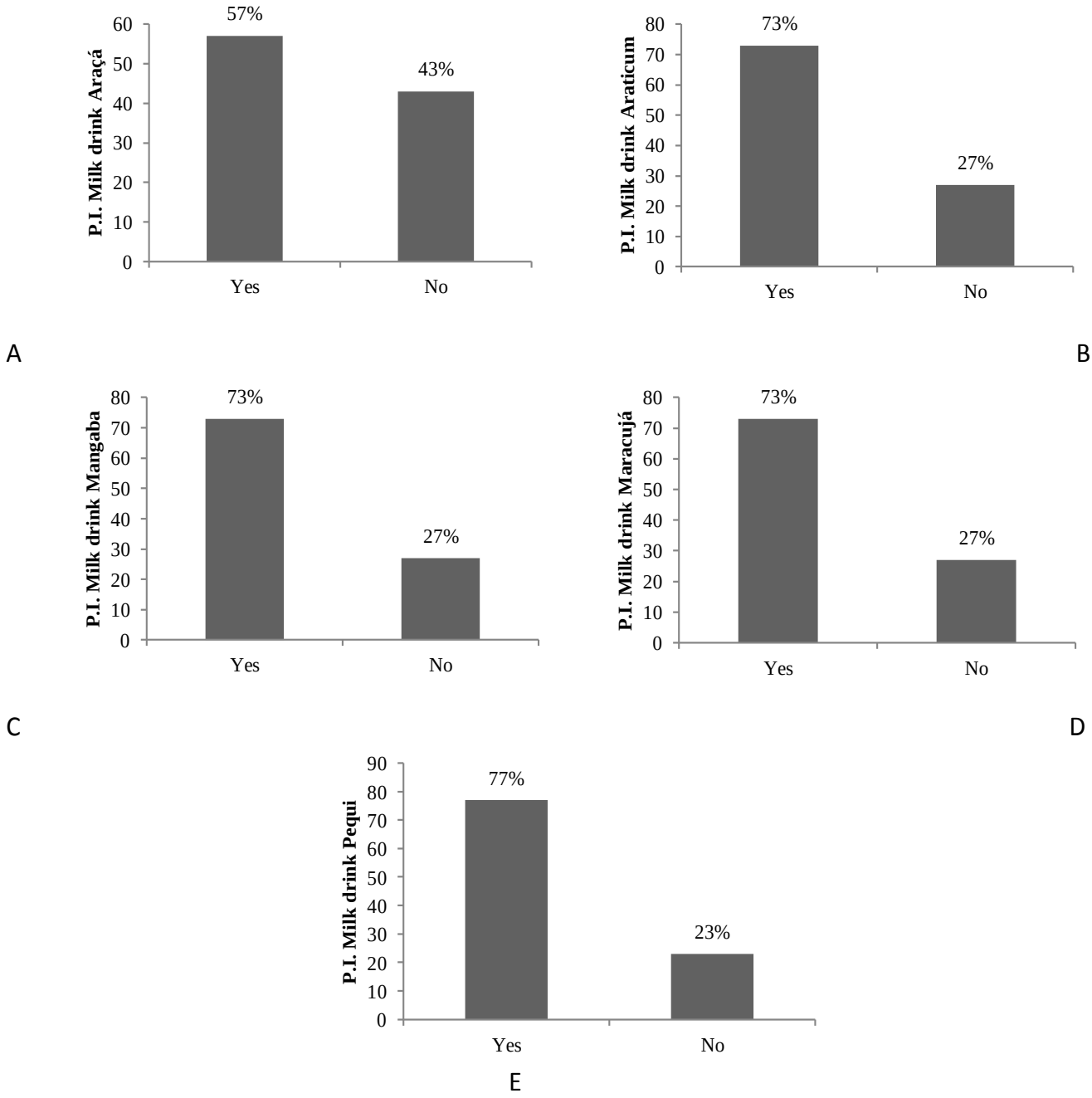

Figure 6. Purchase Intent of milk drinks with increasing levels of fruit pulp. * **P.I. = Purchase Intent** . A. Milk drink flavored with Araçá. B. Milk drink flavored with Araticum. C. Milk drink flavored with Mangaba. D. Milk drink flavored with Passion Fruit. E. Milk drink flavored with Pequi.

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