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Preliminary investigation of the spontaneous lacto-fermentation of cabbage (*Brassica oleracea var. capitata*) grown in northern Algeria

Etude préliminaire de la lactofermentation du chou (*Brassica oleracea var. capitata*) cultivé dans le nord algérien

Djaouida Abbas¹, Wafa Helal¹, Adiba Benahmed Djilali² and Salem Benamara^{1*}

¹Department of Process Engineering, Faculty of Technology, University M'hamed Bougara, Boumerdès, 35000, Algeria

²Faculty of Biological and Agricultural Sciences, University Mouloud-Mammeri, Tizi-Ouzou, 15000, Algeria

*Corresponding author: sbenamara2001@yahoo.fr

Abstract

The present paper reports, for the first time to our knowledge, on the preliminary investigation of the spontaneous lacto-fermentation of cabbage (*Brassica oleracea*, var. *capitata*) grown in northern Algeria. The lacto-fermentation process applied on different cabbage samples was conducted at 20 °C during 21 days and was analysed in terms of physicochemical and microbiological analysis. Among the different fermented cabbage samples, that with the salt concentration of 3% (w/w) gives the most interesting results regarding lactic bacteria development. The thin layer chromatography reveals that the final fermented cabbage contains principally lactic and succinic acids. The total acidity, pH and °brix vary with time according to a linear law ($R^2 \geq 0.98$), whereas the growth of different species of microorganisms during lacto-fermentation follows different kinetics according to the bacterial species. The obtained sauerkraut seems to be free from pathogenic germs (total coliforms, staphylococcus and fecal streptococcus), lactic acid bacteria remaining very dominant compared to the other species present. Given the functional properties of lacto-fermented food products rich in probiotics, these results may contribute to their popularization in North Africa, Algeria in particular.

Keywords: Cabbage (*Brassica oleracea var. capitata*), lactic acid bacteria, lactofermentation, sauerkraut.

Résumé

Le présent article rend compte, pour la première fois à notre connaissance, d'une étude préliminaire sur la lacto-fermentation spontanée du chou (*Brassica oleracea*, var. *Capitata*) cultivé dans le nord de l'Algérie. Le processus de lacto-fermentation appliqué sur différents échantillons de choux a été conduit à 20 ° C pendant 21 jours et a été analysé sur le plan physico-chimique et microbiologique. Parmi les différents échantillons de choux fermentés, celui avec une concentration en sel de 3% (m/m) donne les résultats les plus intéressants concernant le développement des bactéries lactiques. La chromatographie sur couche mince révèle que le chou fermenté final contient principalement des acides lactique et succinique. L'acidité totale, le pH et le °brix varient avec le temps selon une loi linéaire ($R^2 \geq 0,98$), alors que la croissance de différentes espèces de microorganismes au cours de la lacto-fermentation suit des cinétiques différentes selon l'espèce bactérienne. La choucroute obtenue semble être exempte de germes pathogènes (coliformes totaux, staphylocoques et streptocoques fécaux), les bactéries lactiques restant très dominantes par rapport aux autres espèces présentes. Compte tenu des propriétés fonctionnelles des produits alimentaires lacto-fermentés riches en probiotiques, ces résultats peuvent contribuer à leur vulgarisation en Afrique du Nord, l'Algérie en particulier.

Mots clés : Chou (*Brassica oleracea* var. *capitata*), bactéries lactiques, lactofermentation, choucroute.

Introduction

In some parts of the world, fermented foods continues to arouse consumer interest, mainly due to their crucial role in providing food security and health benefits (Uran et al., 2017), including anti-oxidant, anti-microbial, anti-fungal, anti-inflammatory, anti-diabetic and anti-atherosclerotic activity (Şanlıer et al., 2019). Chilton et al. (2015) recommend to the health authorities around the world to include fermented foods in food guides. There are several mechanisms through which fermented foods may exert beneficial effects, among which the presence of probiotic microorganisms like lactic acid bacteria (Dimidi et al., 2019).

Globally, the fermentation process of foods is of two types: spontaneous fermentation by natural microflora present in foods, and fermentation by starter cultures that are added into raw materials.

Spontaneous fermentation is still widely used in traditional household production of fermented pickles in Africa, Asia, Europe, and Latin America (Frias et al., 2017). It is also worth noting that that spontaneous natural fermentation of sauerkraut can occur at lower

sodium levels, considering FDA guidance on the voluntary reduction of sodium in foods (Khanna, 2019). It was reported that, compared to started fermentation, the spontaneously fermented cabbage juice presents the highest intensity of harmonic taste, acceptance of taste, acceptance of odour and flavour (Károvičová & Kohajdová, 2003). In addition, partial or complete elimination of α -galactosides, tannins, phytic acid, and trypsin inhibitory activity was obtained in kidney beans through such fermentation (Frias et al., 2017). However, it is important to take into account the risk associated with the use of spontaneous fermentation, namely implantation/domination of microbial strains dangerous for the human health (Cappozzi et al., 2017).

Despite the health benefits of lacto-fermented plant foods, especially sauerkrauts, these preparations are unfortunately not popularized in North Africa, Algeria in particular. The present paper reports, for the first time to our knowledge, on the preliminary investigation of the spontaneous lacto-fermentation of cabbage (*Brassica oleracea*, var. *capitata*) grown in northern Algeria.

Materials and methods

Plant material

The cabbage (*Brassica oleracea* var. *capitata*) used was cultivated in the region of Boumerdès and bought at the market in the town of Boumerdès (50 km eastern Algiers) and was immediately subjected to fermentation. It is well-known that this vegetable, consumed widely in fresh or sour forms, has an economical and nutritional importance, considering its high content in polyphenols (Jakobek et al, 2018).

Preparation of samples to lacto-fermentation

The stainless steel bench, containers and knife are thoroughly disinfected. The cabbages are washed under running water and then cut into strips 2-3 mm wide. The cabbages thus cut are mixed with cooking salt in proportions which remain to be determined. A 400 g quantity of this mixture is packed tightly into each plastic container used so that the cells burst and the cell juice begins to flow. The mixture is covered with approximately 150 mL of previously boiled and cooled water and then with whole cabbage leaves as a second protective barrier. Finally, the container was closed with its lid and oven heated at 20 ° C for a fixed period.

Physicochemical monitoring of the fermentation process

Influence of salt concentration on final pH value

Lactic acid bacteria are known to be generally able to grow readily in most food substrates and lower the pH rapidly to a point where competing organisms are no longer able

to grow (US National Research Council, 1992). *Leuconostocs* and lactic streptococci generally lower the pH to about 3.5 to 4.5, before inhibiting their own growth (US National Research Council, 1992). In addition, temperature and salt content were reported to significantly influence the fermentation process (Drašković et al., 2018).

Six vegetable samples were prepared as described above, using the salts concentrations of 1.5, 3, 4.5, 5.5, 7.5 and 8.5 % of the total weight of the plant product (w/w) in each container. All samples were then placed into the oven at a constant temperature of 20 °C. The variation of pH was monitored during 21 days, knowing that the optimum temperature for sauerkraut fermentation is around 21 °C (FAO, 1998).

Variation of total acidity, pH and °Brix during lacto-fermentation

The optimal salt concentration established from the previous section was used to monitor the variation of three quality criteria (total acidity, pH and ° brix). For this, seven samples of salted cabbage were subjected to fermentation at 20 °C, using, as previously, thermostatically controlled oven. The variation of the three criteria was determined at different time intervals, for 21 days.

The sample corresponding to the 21st day of fermentation was analyzed for its qualitative composition in organic acids (lactic, succinic and citric acids) using paper chromatography (Whatman filter N°1). The latter is considered to be the simplest and most widely used of the chromatographic techniques (Aryal, 2020). The mixture from butanol-1 (60 mL), acetic acid (15 mL) and distilled water (25 mL) was employed as solvent while bromophenol blue was used as developer. The R_f value was then calculated for each considered organic acid.

Microbiological monitoring of lacto-fermentation process

Along with the physicochemical control of lacto-fermentation process, a microbiological monitoring was also carried out, namely: i) influence of the salt concentration on the microbial load, ii) variation of the microbial load during the lacto-fermentation process, according to procedures described by Bourgeois and Larpent (1996), and iii) isolation and partial biochemical identification of lactic acid bacteria, according to procedures reported by several authors (Bourgeois & Larpent, 1996; Guiraud and Rosec, 2004; Guiraud, 2003; Delarras, 2007).

Results and discussion

pH versus salt concentration

The variation of pH on function of salt concentration is presented in Figure 1.

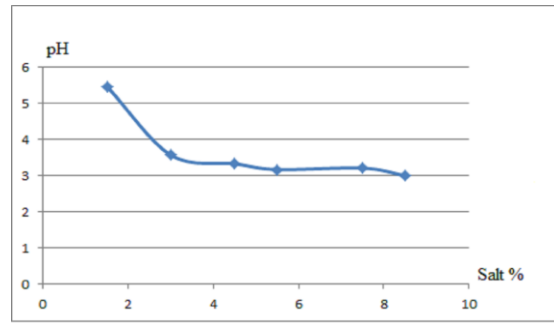


Figure 1: pH versus salt concentration/Variation du pH en fonction de la concentration en sel. As can be seen, the salt concentration of 3 % (w/w) seems to be the most appropriate to reach the desirable pH value (3.56). The findings are in concordance with those naturally extended from such process. In fact, it is established that at the last step of lacto-fermentation, takes place the growth of *Lactobacillus planarum*, producing still more lactic acid and lowering the pH to below 4.0 (Recommendations for Safe Production of Fermented Vegetables, 2009). Thakur and Kabir (2015) have found that, compared to other salt concentrations, sauerkraut with 2.5% salt possessed desirable pH value (3.6), among other analyzed parameters. So, in our case, the salt concentration of 3% (w/w) was used for the rest of the investigation.

Variation of pH, total acidity and °brix during lacto-fermentation process

The variation of pH, total acidity and °brix during the observation period of 21 days is shown in Figure 2.

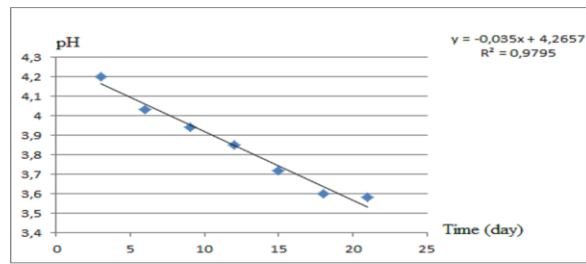
For the three parameters, the data appear to be adequately described by a zero-order kinetics, with R² values of about 0.98 for pH (Figure 2a), 0.93 for total acidity (Figure 2b) and 0.99 for °brix (Figure 2c). In addition, the opposite rate constants of pH (-0.035) and total acidity (0.032) suggest a strong interdependence between the two parameters, recalling in this regard that during lacto-fermentation, the lactic acid concentration should gradually increase, thus causing a drop in pH value.

The qualitative composition in organic acids of sauerkraut sample (after the 21 days fermentation) is shown in Table 1. From these data, it can be observed that lactic and succinic acids appear to be the dominant organic acids formed during the lacto-fermentation. This result is concordant with that reported about the increase of lactic, citric and succinic acid contents, among others, during the fermentation of apple juice (Melini et al., 2019).

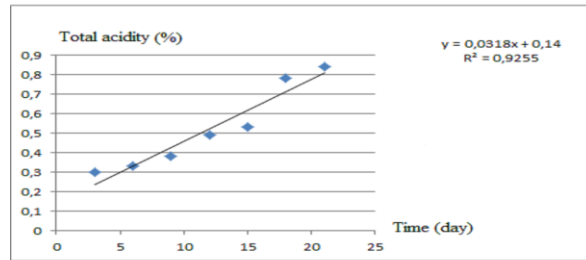
Microbiological results

Influence of the salt concentration on the microbial development

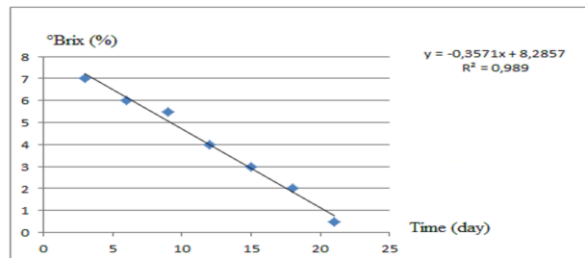
The influence of the salt concentration on the microbial load is shown in Figure 3.



(a)



(b)



(c)

Figure 2: Variation of pH, total acidity and °brix during lacto-fermentation process at 20 °C/Variation du pH, de l'acidité totale et du °brix durant le processus de lactofermentation à 20 °C.

Table 1 : Rf values of organic acids revealed by paper chromatography/Valeurs du Rf des acides organiques révélés par la chromatographie sur papier.

Type of acid	Rf value
Citric	0.64
Succinic	0.82
Lactic	0.85

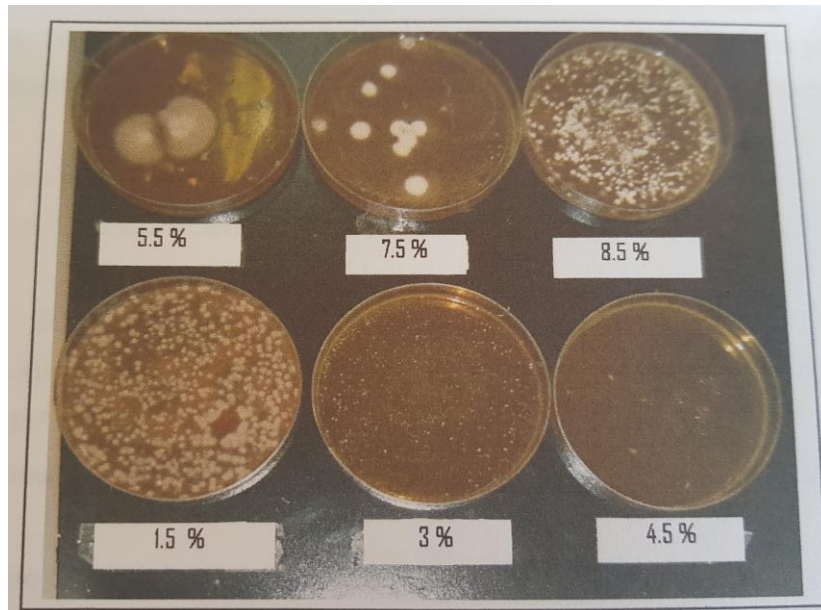


Figure 3: Effect of salt concentration on microbial growth at 20 °C/Effet de la concentration en sel sur le développement microbien à 20 °C.

From this figure it becomes clear that the salt concentration of 3 % (w/w) gives the best results by promoting only the development of lactic acid bacteria. This confirms the previous results regarding the effect of the salt concentration on the final pH of the fermentation medium. Obtained results are in conformity with well-known sauerkraut preparation procedure, namely the presence of not less than 2% nor more than 3% salt (Vegetable Fermentations, n.d).

Variation of microbial growth during the lacto-fermentation process

The variation of microbial growth during the lacto-fermentation process is presented in Figure 4.

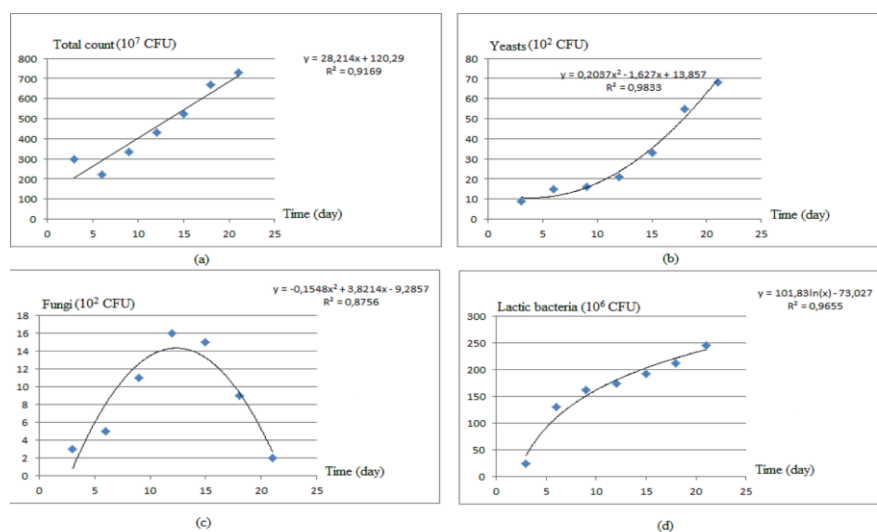


Figure 4: Microbial growth versus time/Développement microbien en fonction du temps.

As can be seen, the growth of different species of microorganisms does not follow the same kinetics. In fact, this is described by a linear law for total germs (Fig. 4a), a polynomial law for yeasts (Fig. 4b) and molds (Fig. 4c) and a logarithmic law for lactic bacteria (Fig. 4d). In all cases, the shapes of the curves do not correspond to the generally accepted model characterized by the presence of four phases (lag phase, log phase, stationary phase, and death phase). The latter phase was observed in the case of fungi which are furthermore undesirable in sauerkraut (Fig. 4c). While there is a presence of other types of microorganisms in the final fermented product, the lactic acid bacteria remain highly dominant.

3.3.3. Isolation and partial biochemical identification of lactic acid bacteria

The microscopic observations (not presented here) of the isolated lactobacillales on MRS agar medium and lactococcus on M17 agar media suggest that these two genera of lactic acid bacteria are responsible, at least in part, for the lacto-fermentation of cabbage. Obtained results are in agreement with literature data. In this context, Zabat et al. (2018) have demonstrated that after two days of cabbage fermentation, the most abundant bacteria present are of the Lactobacillales order and that these bacteria are indicators of successful fermentation.

In the case of bacteria isolated on MRS agar, the results of their partial biochemical identification are summarized in Table 2. From data, the investigated lactic acid bacteria meets the biochemical characteristics of *lactobacillus plantarum*. It is also worth noting that this species is widely used in food industry as probiotic microorganism and/or microbial starter ((Behera et al., 2018).

Conclusion

The obtained results show that it is quite possible to produce in Algeria and throughout the region of North Africa sauerkraut using spontaneous lacto-fermentation. This presupposes meeting the appropriate fermentation conditions in terms of temperature and salt concentration, among others. Considering the functional properties of lacto-fermented plant products, obtained results may contribute to their popularization in previously cited countries. Finally, taking into account these encouraging results, an in-depth study is desired for a better optimization of the lacto-fermentation process (as for example the interest of applying or not a preliminary blanching of cabbages before fermentation), considering the requirements in terms of food sanitation.

Table 2. Partial biochemical identification of lactobacillus genus/Identification biochimique partielle du genre lactobacillus.

Test	Result	Genus	Species
		Lactobacillus	Pantarum
Anaerobic conditions	+	+	+
Mobility	-	-	-
Nitrate reductase	-	-	-
Resistance at 15 °C	+	+	+
Resistance at 40 °C	+	+	+
Resistance at 63 °C	-	-	-
Gelatinase	-	-	-
Indole	+	+	+
Catalase	-	-	-
Oxidase	-	-	-
Lactose	+	+	+
Saccharose	+	+	+
Citrate	-	-	-
Gas	-	-	-
H ₂ S	-	-	-
VP test (acetoin)	-	-	-
MR test	+	+	+
Lysine	+	+	+
Arginine	-	-	-
Indole:			
Urease	-	-	-
TDA	-	-	-

VP = Voges-Proskauer, MR = Methyl red, TDA = tryptophan deaminase.

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