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WORKING UP A LACTOFERMENTED VEGETABLE PRODUCT

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The combination of lactofermentation and enzyme-treatment (Rohament-PL as endo-polygalacturonase and the mixture of Rohament-PL and Rohalase 7069 as cellulase) of sliced carrot and orange juice resulted in a homogeneous product, with pleasant organoleptic features, after 18 h fermentation period. Two ways of inoculation were applied with *Lactobacillus plantarum.* The addition of Rohament-PL, even at 150 mg kg^{-1} concentration, simultaneous inoculation with *Lactobacillus plantarum* (circumstances: 28 °C, 80 r.p.m. shaking) promoted the growth of lactobacilli. By 42 h fermentation time LAB count increased up to $3.2–4.8\times10^9$ cm^{-3}. Furthermore the surface colour of the samples was more intensive (higher L-, a- and b-values) than without *Lactobacillus plantarum* inoculation. The application of Rohament-PL (50–100 mg kg^{-1}) resulted in a homogeneous carrot puree, the combination of Rohament-PL (150 mg kg^{-1}) and Rohalase 7069 (150 mg kg^{-1}) in the process gave a more fluid product, as proved by the lower specific viscosity values.

Keywords: lactofermentation, pectolytic enzymes, LAB count, specific viscosity

The preservation of vegetables by fermentation began before recorded history. Generally, lactofermented vegetable juices improve digestion and they have a regulating effect on the gastric acid (BUCKENHÜSKES & GIERSCHNER, 1987). Extensive reviews are available on the fermentation of sauerkraut, olives and cucumbers (FLEMING & MCFEETERS, 1981). Fermentation of vegetables decreases the nitrate content to about 10% of the original amount (ANDERSSON, 1986). The formation of iron absorption-promoting factors in lactic acid-fermented vegetables was proved (SVANBERG & LORRI, 1997). SULC (1984) suggested the application of a pectolytic enzyme preparation (Rohament P) to the mashed vegetables before the lactofermentation.

In recent years there has been an increase in consumer demand for foods that are only minimally processed without any additives, so the preservation of these type of products present unique challenges to food microbiologists and food technologists (BREIDT & FLEMING, 1997).

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The objectives of the following work were to develop a fresh type product without salt addition, with combination of lactofermentation and enzyme addition. The microbial flora was monitored during the fermentation, the lactofermented products were qualified by measuring the physical (specific viscosity, surface colour) characteristics just after the fermentation process and during cold storage (5 °C) as a function of enzyme concentration.

The realization of the experiments began in the frame of MOE programme as a grant in ATO-DLO (Wageningen).

1. Materials and methods

1.1. Product preparation and processing

Carrot as main component or carrot in combination with orange juice (fresh carrot and pasteurized orange juice without preservatives were purchased in food-shop) were applied in the model experiments (nine fermentations were performed with LAB). Processing of the raw materials into a lactofermented puree comprised the following operations: steam peeling, slicing, enzyme addition, evacuation, inoculation with *Lactobacillus plantarum*, incubation, heating up to 45 °C, chilling, homogenization and storage in cold room at 5 °C.

Carrots were selected and washed, all contaminated parts were removed. The roots were steam peeled at 9 bars for 40 s in a steam peeler machine. Carrots were sliced using Hallde machine, type RG-200 to an average size of 2 mm×2 mm. The components were measured into 500 ml sterilized bottles:150 g of sliced carrot + 300 ml of water or 150 g of sliced carrot + 50 ml of orange juice + 200 ml of water were mixed. For the enzymatic process Rohament PL was added into the samples (50, 100, 300 and 600 mg kg^{-1} for carrot weight) and Rohament PL and Rohalase 7069 were combined, too, in various concentrations (50 mg kg^{-1} Rohament-PL + 50 mg kg^{-1} Rohalase, 150 mg kg^{-1} Rohament-PL + 150 mg kg^{-1} Rohalase).

Rohament PL (Röhm Enzyme Gmbh) is a special pectolytic enzyme preparation for the maceration of fruit and vegetable tissues. It contains almost exclusively endo-polygalacturonase, its enzyme activity is 28000 PGU mg^{-1} (E.C. 3.2.1.15.). It is particularly suitable for the production of fruit and vegetable purees. The other enzyme preparation was Rohalase 7069 (Röhm Enzyme Gmbh). It has a special cellulolytic activity of 1404 CU mg^{-1} (E.C. 3.2.1.4.).

The samples were evacuated in middle vacuum in an evacuator (Shiedam) before inoculation to exhaust the oxygen from the plant material in order to diminish of oxidation reaction in the sample and to infiltrate the enzyme into the carrot pieces.

For the fermentation experiments a *Lactobacillus plantarum* strain (m 33) was applied. This homofermentative microorganism was isolated from olives in Spain. Two methods were used for the preparation of inoculum. In the first case, the LAB cells were grown in MRS media and in the late exponential growth phase were washed with buffer solution (2 mM K_2HPO_4 and 2 mM KH_2PO_4, mixture 1:1, pH = 6.2), the cells were

centrifuged twice (10000 r.p.m. for 20 min), resuspended and 1–1 cm^3 of cell suspension was added to each bottle in the first, second and third fermentations. In this way the initial LAB count of the samples before fermentation varied between 2.0×10^5–3.0×10^6 CFU ml^{-1}.

In the second method 200 cm^3 of heat-treated (121 °C 20 min), sterile carrot puree (pH was adjusted with NaOH to 6.1) was inoculated with 2 cm^3 LAB cell suspension (24 h). From the fourth to ninth fermentation this puree was applied as an inoculum for the samples so the initial LAB counts were in the range of 2.5×10^5–1.5×10^7 CFU ml^{-1} in the bottles.

Samples without LAB inoculation were also prepared and incubated (spontaneous fermentation occured).

Two temperatures were applied (20 °C, 28 °C) for the incubation, the bottles were shaken (GALLENKAMP Incubator) at 80 r.p.m., or not shaken. Incubation time was 18 or 42 h for the lactofermentation and enzyme processes.

At the end of the lactofermentation all the samples were heated up in two steps, at first up to 45 °C, for 60 min in order to increase the activity of the added pectolytic enzyme and then the bottles were pasteurized at 80 °C for 30 min for enzyme inactivation.

The fermented samples were homogenized with a kitchen machine (Type: MOULINEX MASTERCHEF 20) for 20–20 s then the bottles were stored in cold room at 5 °C.

1.2. Physical analysis

The pH of the samples was determined at each sampling time with a glass electrode.

The surface colour of the fermented carrot puree was measured with a MINOLTA Tristimulus Colorimeter equipped with a reflection optical system. The change of *L*-(brightness), *a*-(red-green chromaticity) and *b*–(blue-yellow chromaticity) values were determined just following the fermentation-homogenization and during the storage time (11 days). Each datapoint is presented as the mean of three measurements.

The specific viscosity of the fermented carrot puree samples was measured by a rotation viscosimeter, type BROOKFIELD SYNCHRO-LECTRIC, following the homogenization.

1.3. Microbiological analysis

Total aerobic mesophiles were enumerated on plate count agar (PCA, Oxoid) after 3 days of incubation at 25 °C. Counts of lactic acid bacteria (LAB) were performed on Man-Rogosa-Sharpe (MRS) agar after incubation for 2 days at 30 °C. Moulds and yeasts were enumerated on Malt-extract agar after 3 days at 22 °C. In each case poured plate method was applied.

2. Results and discussion

2.1. Inoculation

Both ways of inoculation (LAB cell suspension and the carrot juice preinoculated with LAB cells) were effective in the fermentations. The various amounts of inoculum did not cause significant differences in the final LAB counts.

2.2. pH measurements

Initial pH of the samples varied according to the components, the change of pH in the bottles during fermentation is summarized in Table 1.

2.3. Colour measurements

The effect of the lactofermentation and enzyme addition to the surface colour was also studied.The *L* (brightness)- *a*(red-green chromaticity)- and *b*(blue-yellow chromaticity) values were determined just after the homogenization (0 day storage time) and during the storage too, until eleven days.The data are summarized in Table 2.

The inoculation with *Lactobacillus plantarum* and the application of endo-PG simultaneously in the process resulted in an intensive orange colour, which was stable during storage time (11 days).

Table 1. Change of pH during fermentation

Fermentation time (h)	150 g carrot + 300 cm^3 water		150 g carrot + 50 cm^3 orange juice + 200 cm^3 water	
	without LAB	with LAB	without LAB	with LAB
0	6.4–6.5	6.4–6.5	4.6–4.7	4.6–4.7
18	4.8–4.9	4.1–4.2	4.4–4.5	3.5–3.7
42	4.2–4.3	3.2–3.4	3.8–3.9	3.0–3.2

Table 2. Change of surface colour during storage

Parameters	Inoculated, 600 mg kg^{-1} Rohament PL Storage time (days)				Not inoculated, 600 mg kg^{-1} Rohament PL Storage time (days)			
	0	4	6	11	0	4	6	11
L	39.6 ± 0.1	39.7 ± 0.1	40.7 ± 0.2	40.7 ± 0.1	38.5 ± 0.1	37.4 ± 0.4	37.7 ± 0.3	38.2 ± 0.1
a	9.7 ± 0.05	9.9 ± 0.06	9.5 ± 0.2	9.3 ± 0.1	8.7 ± 0.02	8.6 ± 0.4	7.7 ± 0.1	7.3 ± 0.06
b	20.8 ± 0.3	21.1 ± 0.1	20.2 ± 0.1	19.8 ± 0.1	18.2 ± 0.2	17.6 ± 0.8	15.3 ± 0.4	14.6 ± 0.2

Table 3. Specific viscosity of carrot puree after 18 h of lactofermentation at various endo-PG concentration

Rohament – PL (mg kg^{-1})	Specific viscosity (cp)
0	30 ± 9
50	540 ± 21
100	570 ± 15
300	534 ± 12
600	565 ± 17

Table 4. LAB counts after 18 h of fermentation time as a function of Rohament-PL concentration

Rohament-PL (mg kg^{-1})	LAB counts (CFU ml^{-1})	
	0 h	18 h
0	5.6×10^6	4.1×10^8
50	3.2×10^6	5.8×10^8
100	3.2×10^6	1.6×10^9
300	5.8×10^6	1.5×10^9
Control[a]	$<10^2$	1.1×10^7

[a] no added LAB inoculum

All the three parameters were higher – just after the homogenization and during the storage time too – in those samples where *Lactobacillus plantarum* was inoculated and endo-PG was applied simultaneously in the process. After 11 days of storage the colour loss was dramatically quick in the spontaneous fermented samples, while the samples inoculated with *Lactobacillus plantarum* had much more stable colour. This result is similar to those, where fermentation significantly increased lightness (*L* values) and yellow chromaticity (*b* values) in carrot chips compared to unfermented chips (SLINDE, 1993).

2.4. Specific viscosity

Following the heating up and homogenization of the fermented carrot puree samples, the specific viscosity was also determined. Increasing the endo-PG (Rohament-PL) concentration the specific viscosity also increased up to 100 ppm Rohament PL. Change of specific viscosity (three replicates) is summarized in Table 3.

2.5. Effect on microbial flora

The initial population of the raw material was investigated before inoculation: total aerobic mesophiles were 1×10^3–2×10^3 CFU ml^{-1}, the number of lactic acid bacteria and yeasts were less than 10^2 CFU ml^{-1}, respectively. After 18 h-fermentation period (in the inoculated samples with *Lactobacillus plantarum*) the LAB count increased up to 10^8–10^9 CFU ml^{-1}. This result is in the same order of magnitude as other researchers

have found (SLINDE, 1993). The LAB count seemed to be promoted by the addition of Rohament-PL up to 100 ppm concentration, this was a tendency not a significant difference. Data represent three replicate samples, SD was lower than 0.5 log cycle.The results are shown in Table 4.

In this phase aerobic mesophiles (7×10^2–9×10^2 CFU ml^{-1}) and yeasts were also detected, 2×10^2–2×10^3 CFU ml^{-1}, in the samples. In the literature it is also known that some pigmented yeasts can grow under such circumstances (FLEMING et al., 1995).

When Rohament-PL and Rohalase were added in combination (150 mg kg^{-1} + 150 mg kg^{-1}) the number of *Lactobacillus plantarum* in carrot suspension during fermentation showed the same tendency as Rohament-PL (100 mg kg^{-1}) alone after 18 h fermentation time (data not shown). When the lactofermentation was stopped after 42 h, LAB count did not increase further significantly, and aerobic mesophiles and yeasts were not detected, probably because of the low pH.

Samples prepared by *Lactobacillus plantarum* inoculation, without enzyme addition reached a lower final CFU number, possibly because the maceration of carrot cells provides a higher amount of substrate for the lactofermentation.

The experiments proved that the combination of lactofermentation and enzyme-treatment of the mixture of carrot and orange juice resulted in a homogeneous carrot puree with a good taste and colour.

3. Conclusion

On the basis of our preliminary experiments it seemed that the most appropriate fermentation time would be 25–30 h at 28 °C (shaking at 80 r.p.m.) in order to get a product with good taste and colour. Further experiments should be done to prove the safety of this minimally processed product.

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