POTENTIAL OF FERMENTED SPINACH EXTRACTS AS A NITRITE SOURCE FOR MEAT CURING

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Abstract - There is an increasing interest in the use of natural sources of nitrite for curing meat. Vegetables are good sources of nitrate and it can be used to cure meat if a starter culture that reduces nitrate to nitrite is provided. Fresh and dried spinach were screened as potential sources of nitrate and the ability of *Staphylococcus carnosus* to convert nitrate to nitrite was tested. Conversion efficiency of nitrate to nitrite was higher in fresh spinach than in dried spinach. The results of a kinetic study revealed that the maximum conversion using 50g/L fresh spinach by *S. carnosus* was obtained after 20h fermentation, resulting in 150mg/l nitrite. Freeze-dried supernatant of the fermented spinach (VegN) was added to minced pork meat as curing agent and compared with synthetic nitrite ($NaNO_2$) (SynN) and no nitrite in terms of color and lipid stability of fresh and cooked meat during time of display. No clear differences were observed for color and lipid oxidation between VegN and SynN treatment. Residual nitrite was significantly lower in the VegN treated pork meat compared to the SynN treatment.

Key words: Spinach, *Staphylococcus carnosus,* stability

- INTRODUCTION

Nitrites are very important in meat curing because of their unique properties in color formation, antioxidant activity, typical cured flavor and bacteriostatic effect [1-3]. However, there is concern about the safety of nitrite as it is known to react with secondary amines leading to the formation of nitrosamines, many of which have been found to be carcinogenic [4, 5]. The challenge for the meat industry is thus to find strategies to reduce residual nitrite in cured meat as this can directly reduce the intake of nitrites from cured meats [2].

The use of vegetables as a source of nitrate is being exploited because of the vast amount of nitrates that has been found in certain vegetables. Some vegetables such as celery, spinach, radish and lettuce have been found to contain more than 2500mg/L nitrate [6]. Celery juice and celery powder is being frequently tested as a pool for nitrate because of its high compatibility with processed meat products since it does not impart any taste and vegetable flavor to the meat [7]. However, other vegetables such as spinach, grown in high amounts in a lot of countries also have potential to be used.

In order to convert nitrate to nitrite, a nitrate reducing enzyme is needed and this can be found in some microorganisms. The most common microorganisms used are some Staphylococcus strains which include *S. xylosus, S. carnosus. subsp. carnosus, S. carnosus subsp. utilis, S. vitulinus* and *S. equorum* [7,8].

Research on production of products containing natural nitrite is still in its infancy. The safety, regulation, manufacture and quality of these products still needs to be clarified.

The objective of this study was to prepare a spinach powder by fermentation with *S. carnosus* that can be used as a source of vegetable nitrite to cure pork.

- MATERIALS AND METHODS

Screening study of the potential of spinach as nitrite source

Different amounts of spinach (increasing from 0-500g/L), fresh or dried (at 105°C), were added to a brine solution containing 8% glucose, with (3%) or without yeast extract as nitrogen source. A positive control was included using 100 mg/l $NaNO_3$ as nitrate source instead of spinach, with the same brine composition. After sterilizing the medium, *Staphylococcus carnosus* (LMG26366) was added at 10^6 CFU/ml. Incubations were done for 72 hours under aerobic static conditions at 37°C. Samples were taken 0, 24 and 72 hours to measure pH and perform nitrate/nitrite analysis [9]. All incubations were done in duplicate.

Kinetic study

Fresh spinach (50g/L) was added in 100ml brine solution (8% glucose solution) and sterilized. Inoculation was done with *S. carnosus* at 10^6 CFU/ml. Incubations were done for 26 hours under aerobic static conditions at 37°C. Samples were taken every 2 hours for growth, pH and nitrate/nitrite analysis [9]. This was done in duplicate.

Processing of cured pork

The supernatant of fermented fresh spinach was prepared in a similar way as described in the kinetic study. After 20 hours, fermentation was stopped and the supernatant was freeze-dried. Three treatments for curing pork were considered: NoN = no addition of nitrite ; SynN = 80 mg/l nitrite supplied using synthetic nitrite curing salt (containing 0.06 % nitrite and 99.4% NaCl); VegN : 80mg/l nitrite supplied by the freeze-dried supernatant of the fresh spinach fermentation. In all treatments, 0.5g/l sodium ascorbate was added, and the NaCl concentration was made equal to 13.25 g/l.
Pork was minced and frozen overnight. After thawing, the dry ingredients were dissolved in water (5%), sprinkled over the minced pork and thoroughly mixed. The mixtures were subsequently shaped into patties of 50 g. For each treatment, ten patties were made. Patties were displayed for 48 hours in an illuminated cabinet at 3 °C. Color measurements were taken once per hour in the first 5 hours and then after 24 and 48 hours using a Miniscan HunterLab XE Plus colour meter. After display, samples were stored vacuum-packed at -18°C for TBARS [10] and residual nitrite [9] analysis. After 24 hours, 3 patties of each treatment were vacuum packed and cooked (70°C, 40 min). After cooling, color measurements were performed on a fresh-cut surface, and samples were displayed in an illuminated cabinet at 3°C for 5days. Samples were stored at -18°C, and used for TBARS [10] and residual nitrite [9] analysis.

- RESULTS AND DISCUSSION

Screening experiment to study the potential of spinach as nitrite source

The bacteria did not grow well in treatments with dried spinach because drying of the vegetables probably destroyed most of the essential nutrients needed for the bacteria to grow.

Sablani [11] showed that oven drying of vegetables causes major changes to the nutritional profile of vegetables. A significantly higher conversion efficiency of nitrate to nitrite in the 72h incubations was observed for fresh spinach (15.9 ± 6.9 %) compared to dried spinach (2.4 ± 1.2 %). Conversion efficiencies in the incubations with $NaNO_3$ were 19.9 ± 2.1 % after 72h.

Based on these results, it was concluded to continue with fresh spinach in a concentration of 50g/l. The screening experiments also revealed that *S. carnosus* was able to use fresh spinach as nitrogen source, so there was no need to supply additional yeast extract to the 8% glucose solution.

Kinetic study

The pH was reduced from 6.2 to 4.5 for both the incubation with fresh spinach and with $NaNO_3$, after 30h incubation. Maximum growth of the bacteria was observed after 8 hours i.e. 7.5 and 8 log CFU/ml for fresh spinach and $NaNO_3$ incubations respectively. For the control, the maximum conversion of nitrate to nitrite was observed after 6 hours while for the treatment with fresh spinach the maximum conversion was observed after 20 hours (Fig. 1). The differences in time of attaining the maximal conversion of nitrate to nitrite could be attributed to the initial amounts of nitrate present. Fresh spinach added at 50g/L supplied on average 215 mg/L nitrate which is higher than 100 mg/L $NaNO_3$ supplied. This may mean that the bacteria needs more time to work on the higher amounts of nitrate.

Figure 1: Conversion % of nitrate to nitrite for medium with fresh spinach and control medium (n=2)

It was impossible to produce sufficient concentrations of nitrite in the supernatant for applying in meat curing, as the supernatants can only be added to the meat at a low percent. Higher nitrite concentrations are not feasible in the supernatant by batch fermentations, as the nitrate reductase system of the bacteria seems to be inhibited by high levels of nitrite and nitrate. This has also been demonstrated by Krause at al. [5]. For this reason, the supernatant, obtained after 20h fermentation, was freeze-dried to obtain a concentrated powder, making it able to cure a meat product using 80 mg/L nitrite from a vegetable fermented source.

Meat quality measurements

During time of display, both for fresh cured and cooked samples, the VegN resulted in significantly lower L^*-values compared to NoN and SynN, while there was no difference for the a^* value between treatments at the end of display (data not shown).

Lipid oxidation, as measured by TBARS, increased during time of display for the fresh cured samples, with the highest increase observed for NoN. Also after cooking the NoN showed the highest TBARS values (Fig. 2). These difference can be attributed to the well-known antioxidative activity of nitrite [7; 12].

Fig 2: TBA content (µgMDA/g) for NoN, SynN and VegN treated pork patties

Table 1 shows the results for residual nitrite in fresh cured and cooked pork. The results show that immediately after manufacturing of the product, the nitrite decreased by at least 50% in SynN and VegN. Lower values of residual nitrite were observed for VegN than for SynN (Table 1).

Table 1 Residual nitrite (mg/kg) in pork, according to the treatments (n=3)

Treatment	Day 0	Day 2	Cooked
NoN	3.97^{a}	3.87^{a}	3.87^{a}
SynN	$43.03^{b,x}$	$30.09^{b,y}$	35.42^{b}
VegN	21.08^{c}	19.44^{c}	19.34^{c}
SEM	5.66	3.81	4.58

$^{a,\ b}$ means within same column with different superscripts are significantly different ($P < 0.05$); $^{x,\ y}$ means within same row with different superscripts are significantly different ($P < 0.05$)

The decrease of nitrite throughout storage is common and a widely recognized phenomenon [13]. The lower residual nitrite in the VegN treatment could be caused by the lower pH and some additional ascorbic acid and other components derived from the plant extract. Addition of ascorbate also accelerates the depletion of nitrite because ascorbate provides reducing conditions which increases the rate of formation of nitric oxide.

Although lower residual nitrites are desired, some amount is needed to provide microbial safety to the product. Cassens [14] reported that residual nitrite is essential in maintaining cured color and flavor during storage and the amount reported to be sufficient was 5 - 15 mg/L for commercial cured meats in the USA. Several studies have shown that meat products that have been cured using natural nitrite are at greater risk for the growth of foodborne pathogens [15-17]. Because of this microbial safety concern, further studies need to be done to make sure that these products are safe and that initial lethality and sustained inhibition of pathogens is achieved.

- CONCLUSION

Spinach can be used as an alternative for nitrite or a source for nitrate in meat curing. However, fermented spinach supernatant as such has too low nitrite concentrations, so a further concentration is needed. Added to meat in similar concentrations as $NaNO_2$, it exhibited similar effects on colour and lipid stability. However, further studies need to be done to ascertain the safety of these products.

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