Efficacy of ready-to-eat probiotic artichokes in modulating gut microbial parameters in healthy subjects and patients with functional constipation.

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Mutually beneficial relationship between the host and its resident microbiota

‘We feed our microbes, they talk to us and we benefit. We just have to understand and then exploit this.’
(Willem de Vos).

De Vos et al. 2012. Impact of Microbiota in Health and Disease. SelfCare 3(S1):1-68
Commensal bacteria

- support the digestion of fibres and other nutrients
- contribute to energy and substrate supply
- Contribute to the acidification of the gut
- regulate epithelial functions;
- prevent colonization of pathogens in the gut
- regulate the mucosal immune system

**Mechanisms of action of the intestinal microbiome on the gastrointestinal barrier**

Bishoff BMC Medicine. 9:24
Non-digestible carbohydrates fermented in the colon to yield energy for microbial growth and SCFAs.

**Role of SCFA on intestinal functions**

- Trophic effect of intestinal epithelium
- Effect on the differentiation of epithelial cells
- Modulation of ion absorption
- Modulatory effect on glucose metabolism
Gut microbiota manipulation: the use of probiotics

Increase of the relative numbers of “beneficial bacteria” of gut microflora

Acidification of the gut and improvement of the nutritional status of gut epithelium

Strengthen intestinal barrier function and antagonize pathogens
Importance of food in probiotic efficacy

- Probiotics transiently colonize the gut, large populations need to be ingested daily.

- Survival is a strain-related ability but is influenced by the protective action of the carrier.

- Foods help to buffer the probiotic through the gastrointestinal tract.

- Regulate their colonization (presence of prebiotic substances).

- A suitable food is crucial for developing a probiotic.
A DELICIOUS ALTERNATIVE? THE “HORTOBIOTICS”

Drawbacks associated with the consumption of probiotic products

"Milk-based"

Lactose intolerances

Cholesterol-restricted diets

Growing veganism

Consumers’ taste (lack of sensory appeal)
Anchorage of *Lactobacillus paracasei* LMG-P22043 to artichokes

Approved by the Italian Ministry of Health
Efficacy of the probiotic vegetable gastronomy

A portion of artichokes can carry more than 1 BILLION LIVE AND ACTIVE BACTERIA amount comparable or greater than those of milk-based products.
Efficacy of probiotic artichokes in human trials

- Suitability to deliver the probiotic in adequate amounts
- Modulation of intestinal microflora
- Colonization of human gut
- Probiotic artichokes enriched with *L. paracasei*
  - Effect on SCFA production
  - Effect on faecal enzymatic activities
  - Effect on constipation
**CLINICAL EVIDENCES**

*Selection of subjects*: 20 healthy subjects (3 men and 17 women, age 37.8 ± 13.9 years).

Randomized, double-blind human trial in comparison to probiotic-free artichokes (control)

<table>
<thead>
<tr>
<th>Group</th>
<th>Artichokes</th>
<th>Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>A group</td>
<td>Probiotic-artichokes</td>
<td>T0, T1</td>
</tr>
<tr>
<td></td>
<td>Control-artichokes</td>
<td>Tw</td>
</tr>
<tr>
<td>B group</td>
<td></td>
<td>Period 1</td>
</tr>
</tbody>
</table>

**Role of the probiotic strain *Lactobacillus paracasei* LMGP22043 carried by artichokes in influencing faecal bacteria and biochemical parameters in human subjects**

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Artichoke preparation

Ordinary and probiotic artichokes were lightly seasoned with olive oil and packed in identical trays with modified atmosphere to obtain ready-to-eat artichoke products (about 180 g).

Final products had identical shape, texture, and appearance and there was no way to distinguish between the two products.

Probiotic artichokes contained approximately $2 \times 10^{10}$ of probiotic cells per portion (daily-dose)
Probiotic colonization

Presence of *L. paracasei* LMG P-22043 at T₁ only in Group A (6.87 log₁₀ CFU/g)
(80% colonized subjects)

Absence of *L. paracasei* LMG P-22043 at
- To and Tw (Groups A and B)
- After control artichoke intake (T₁, Group B)
The probiotic strain modulates the intestinal flora by increasing the biodiversity of lactic acid bacteria and reducing potential pathogens.

- Modulatory index
- MIPR/CTR indicates caused a shift of microbial counts towards lower numbers of Enterobacteriaceae, E. coli, total Clostridium and higher values of LAB, presumptive lactobacilli and bifidobacteria.
Effect on faecal microbial groups

LAB

log CFU/g

presumptive bifidobacteria

log CFU/g

presumptive lactobacilli

Total *Clostridium* spp.

log CFU/g

*Clostridium* spores

log CFU/g

*E. coli*

log CFU/g

Enterobacteriaceae

Control group

*Probiotic group A*

Total *Clostridium* spp.

log CFU/g

*Clostridium* spores

log CFU/g

*E. coli*

log CFU/g

Enterobacteriaceae

log CFU/g

*Probiotic group A - B*

E. coli

log CFU/g

Enterobacteriaceae

log CFU/g

*Control group*

LAB

log CFU/g

presumptive bifidobacteria

log CFU/g

presumptive lactobacilli

log CFU/g

Control group

*Control group - B*

Log data obtained from T0. P < 0.05
**Genetic diversity of faecal LAB population**

- **T0, H = 0.92**
  - 11 isolates, 4 profiles

- **T15- PR, H = 1.23**
  - 22 isolates, 8 profiles

- **T0, H = 1.22**
  - 21 isolates, 7 profiles

- **T15- PL, H = 1.19**
  - 20 isolates, 4 profiles

\[
H = \sum -\left(\frac{N_i}{N} \times \ln \frac{N_i}{N}\right)
\]

- **N** = total number of isolates
- **N_i** = number of isolates for each REP–PCR profile
Effect of probiotic artichoke intake on biochemical parameters

**Probiotic group A**

Short chain fatty acids

**Control group B**

Faecal enzymatic activity
Disturbances in the gut microbiota may contribute to symptomatology and etiology of functional diseases.

Functional constipation is associated to:
- Reduced levels of Lactobacilli and Bifidobacteria
- Increased levels of Clostridium
- Presence of “minimal inflammation”

New therapeutic approach for constipation could be based on the modulation of intestinal microflora by administering prebiotics and/or probiotics.
8 subjects, 3M/5F age 40±14 yr suffering for mild constipation (Rome II criteria) integrated their usual life style with probiotic artichokes on the base of the

- Colonization by the probiotic strain of the human gut of all volunteers
- Individual response of subjects to the probiotic artichokes regarding the modulation of microbial population
- Overall positive effect on symptoms profile of participants

**Efficacy of probiotic vegetables in a human study**

Microbiological and biochemical analyses of faecal samples;
Gastro-intestinal symptom questionnaire (GSRS) and Bristol form chart compilation
Gastro-Intestinal Symptom Rating Scale

**Abdominal distension**

- Control time vs. Test time
- p < 0.019 Wilcoxon matched pair test

**Increased flatus**

- Control time vs. Test time
- p < 0.001 Wilcoxon matched pair test

**Decreased passage of stools**

- Control time vs. Test time

**Feeling of incomplete evacuation**

- Control time vs. Test time
- p < 0.001 Wilcoxon matched pair test

Bristol form chart result: stool consistency softer in all subjects.
Randomised clinical trial: efficacy of *Lactobacillus paracasei*-enriched artichokes in the treatment of patients with functional constipation – a double-blind, controlled, crossover study

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CNR-ISPA and I.R.C.C.S. ‘Saverio de Bellis’, National Institute of Digestive Diseases

**Patients**

**Inclusion criteria**

- 30 patients - age 19–70 years
- functional constipation
  - Rome II criteria
    - Constipation Scoring System (CSS) (symptom questionnaire)
- GI imaging study < 5 yrs

**Exclusion criteria**

- major abdominal surgery;
- the presence of any concomitant diseases;
- alarming symptoms;
- abnormal laboratory data or thyroid function;
- family history of peptic ulcer, colorectal cancer, or IBD.
Design of the study.
# RESULTS

Colonization: 17/20 subjects

## Symptom profile (GSRS score)

<table>
<thead>
<tr>
<th></th>
<th>Baseline run-in</th>
<th>Control Ordinary artickokes</th>
<th>Probiotic Enriched artickokes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reduced Frequency of evacuation</strong></td>
<td>2.9±1.5(^a)</td>
<td>2.5±1.6(^{ab})</td>
<td>1.9±1.6(^b)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Hard stool</strong></td>
<td>3.0±1.6</td>
<td>1.9±2.0</td>
<td>1.5±2.1</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Feeling of Incomplete evacuation</strong></td>
<td>4.4±17°</td>
<td>3.7±2.3(^{ab})</td>
<td>2.8±2.2(^b)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Stool consistency (*Bristol score*)

![Bristol Score Graph](image)

*P = 0.009*
CONCLUSIONS

Probiotic-enriched artichokes

- reduce GSRS item scores
- improve stool consistency
- Improve microbiological intestinal parameters

Take home message

*The introduction of vegetables enriched with probiotics such as artichokes, but also salads and olives, (hortobiotics) could represent a way to achieve the target “functional diet”*
Final thoughts

Probiotic vegetable gastronomy provides a concrete opportunity to convey probiotic benefits already appreciated by consumers in other market sectors.

The proficient association of the strain with a food carrier rich in fibre can represent a new strategy for favouring a daily supply of probiotics and attracting more consumers to foods fortified with probiotic strains.
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