

# A Review 'Clean Labeling': Applications of Natural Ingredients in Bakery Products

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**Abstract** Clean labeling has been a trend and the term “clear label” incorporates the concept of transparency. Thus, this review aims to search in the literature for a better way to extend the shelf life of bread by using ecologically preservation techniques as alternatives to chemical additives. In spite of modern advances in technology, the preservation of foods is still a debated issue, not only for developing countries but also for the industrialized world. There is a increased interest in biopreservation, aiming to extend the shelf life and enhancing food safety by using natural microbiota and/or antimicrobial compounds. The use of natural preservatives would enable bakeries to market the ‘clean label’ or ‘label friendly’ products. Thus, the free-from trend leads the bakery industry to reconsider the traditional preservation methods and replace chemical preservatives with natural alternatives to guarantee the clean label. This process is based on the tendency in dairy category, which coincide with manufactures that incorporate no artificial/all natural/GMO free/BPA free claims on products. In this sense, consumer awareness of food ingredients and the desire for simple, natural foods have forced food manufacturers to develop products with a clean label appeal. It is therefore critical to consider the implications of developing a clean-label product, taking into account the effects such a change may have on sensory quality and microbiological control, while also maintaining regulatory compliance.

**Keywords:** *clean label, bakery ingredients, natural ingredients*

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## 1. Introduction

Recently, consumers have started to pay greater attention to what is in their food, where it comes from and how it is made. According to Sweetman [1] in Europe, the number of consumers who consider the ingredient list as an important item has risen from 3% between 2011 and 2013 to 78%, and it is now the second most important factor when choosing a product, after price.

Within the food sector, there is a growing back-to-basics attitude. Consumers and manufacturers alike are moving towards ‘less processed’ foods made of easy-to-understand ingredients. This desire for simplicity has been influencing the ‘clean label’ trend [2]. Lately, there has been an increased consumer interest in “clean labels” worldwide, particularly in the U.S. However, there is no legal or regulatory definition for “clean label.” Customers and stakeholders may associate clean labels to foods that are minimally processed, devoid of artificial flavors, colors, synthetic additives and unexpected allergens [3].

The clean label trend is led entirely by consumers, albeit unconsciously. Consumers are increasingly concerned about what is in their food and how it is made,

which drives a demand for ‘clean label’ products. At the same time, the EU legislation requires dietary supplement manufacturers to present clearer and more comprehensive information on their labels. Consumers across Europe say clean label plays a key role in their decision-making. Clean label demand vary by country: UK 37%, Spain 58%, Poland 59%, Italy 65%, Germany 50% and France 46%. This has been leading manufacturers to introduce new clean label supplement products in Europe. The proportion of foods supplements brought to market with clean label claims is 35% in Western Europe and 17% in Eastern Europe [4].

The clean label trend is one of the major factors contributing to the ethical label growth worldwide with US \$165 billion in value sales, which comes from packaged food, soft drinks and hot drinks. A key category among the clean labels is the “no artificial preservatives” pattern, which has been growing at a fast pace in clean label category. Many of these products will be delivered by the dairy industry as ready meals, sauces, dressings and condiments [5].

There are few scientific data to support the rationale by seeking a clean label and little agreement about what a clean label really means; on the other hand the consumer’s interest is a real and growing phenomenon that is driving a large response by the food industry [3].

“Clean label” is relatively new as a food industry term, but the characteristics within that term may appeal to an older demographic market. Thus, this review aims to search in the literature for a better way to extend the shelf life of bread by using ecologically preservation techniques as alternatives to chemical additives. This scenario makes necessary the development of clean-label alternatives.

## 2. Clean Label

The clean label movement first emerged in the UK approximately 20 years ago. Currently, it is particularly prominent in France, Germany, the UK, the US and has gaining force in others key markets [6].

Euromonitor International recently published its latest ingredients data in which the clean label has been a trend in food and beverages, and that tendency has been shown in the ingredients as well. Those components such as natural hydrocolloids has been shown to present good growth rates as well as botanicals, due to the consumers demand for products that includes those ingredients [7].

Despite the increasing market shift towards clean label food products and a large number of different studies that have investigated goods carrying clean label, it is not yet clear what a clean label exactly means. So far, a jointly agreed upon definition or specific regulations/legislations does not exist, leaving the interpretation as rather subjective for consumers and food practitioners. A clear definition of clean label that can improve understanding of consumer perception and behavior, guide manufacturers in food development and communication, and support policymakers' efforts in providing a targeted regulatory framework is needed [8].

A new data released by Innova Market Insights has found that consumers are demanding for shorter and more recognizable ingredients lists and the manufacturers are responding by increasingly highlighting the local and origin of their products. However, with the concern in the lack of a definition for “natural” it still needs for more clarity and specificity [9].

According Canadean [10] there is no one definition of “clean label” so it may mean different things to the people. However, Clean label tends to be closely associated with the following themes or concepts:

- Nature - The closer to nature an ingredient or product is, the more “clean” it is perceived to be.
- Simplicity - Less chemicals and more cupboard friendly. Simplicity and familiarity count.
- Transparency - Where the ingredients come from and how the products are made.
- Processing - The more processed a food or drink is, the less “clean” it is perceived to be.

One useful definition was devised by National Starch in the US: Free from chemical additives; simple ingredient listing (without ingredients that sound chemical or artificial); minimally processed using traditional techniques that are known by consumers and not perceived as being artificial [11]. In the USA, the Food and Drug Administration (FDA) has indicated that “natural” may be used since it is proven to be truthful and not misleading (i.e. the product

does not contain any artificial flavour or flavourings, colouring ingredients, chemical preservatives or any other artificial or synthetic ingredients) also the product and its ingredients are for that reason, minimally processed. The Food Standards Agency (FSA) in the UK also provides similar guidance for the use of the terms “fresh,” “pure,” “natural,” etc., in food labelling [12]. However, both these agencies tend to deal with the use of “natural” in labelling from a marketing perspective, and do not need to care about the full “clean label” definition [13].

Some trends to consider that are currently contributing to the demand for clean label ingredients are discussed below [11].

1. Through the various celebrity chef cookery programmes and advertising by the supermarket groups, consumers are increasingly avoiding foods high in fat, salt, sugar and also foodstuff containing additives.

2. Food labels are increasingly referring to products as local, kitchen-style or home-style, etc.

3. The percentage of consumers who are reading labels is thought to be raising up.

4. The terms ‘natural’ or ‘free from additives’ are constantly appearing on food product labels.

5. Descriptive language on food packs increasingly describes products as ‘pure’ or ‘fresh’.

More than twenty percent of US products tracked in 2014 featured a clean label positioning compared to the seventeen percent in 2013. Significant rises in the use of clean label ingredients have also been tracked by Innova Market Insights, with growing interest in natural sweeteners, such as stevia and monk fruit, natural colours such as those based on spirulina, elderberry, beetroot, and thickeners (i.e tragacanth and gellan gums). Fewer ingredients with more natural flavours and colours and recognizable ingredients are on top of the list of consumer requirements [9].

More recently, surveys suggest that a large majority eighty four percent of Americans purchase foods that have “free from” labels because they believe those are less processed and more natural. In addition, the increasing number of products labeled with negative claims such as “free from preservatives” has been suggested to contribute to consumer's fear of additives [14].

According to a recent study evaluated by Canadean, the companies that are embracing the clean labeling may not be gathering the consumers as they were expecting. The term “clean label” has many definitions among people all around the world (Figure 1). The study found that thirty four percent of consumers do not have an understanding of what the term really means Perhaps the question that should rise would be if the consumers would pay more for clean label products. The study revealed that the majority of U.S. consumers, sixty nine percent, would not pay more than the price of a regular product for a product with a “clean label” claim. Just seven percent of consumers would be willing to pay five percent or more for a product with a clean label claim. “This reluctance to pay more for clean label stems partly from lack of understanding as to what the term means, but also perhaps indicates that consumers believe the ideals of clean labeling should be inherent in a product, and not something you have to pay more to get” [15].

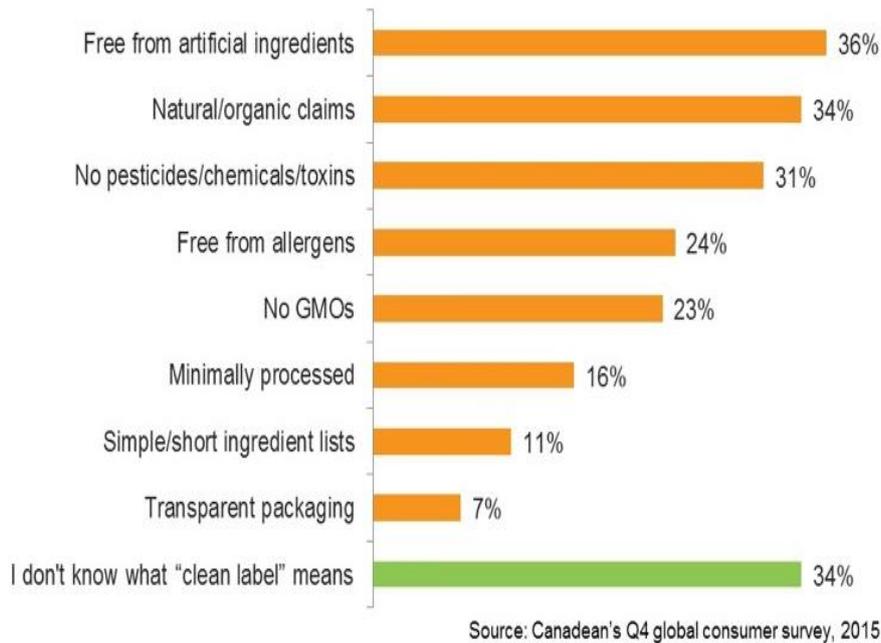


Figure 1. Global: What does the term “clean label” mean to you? (Source: [15])

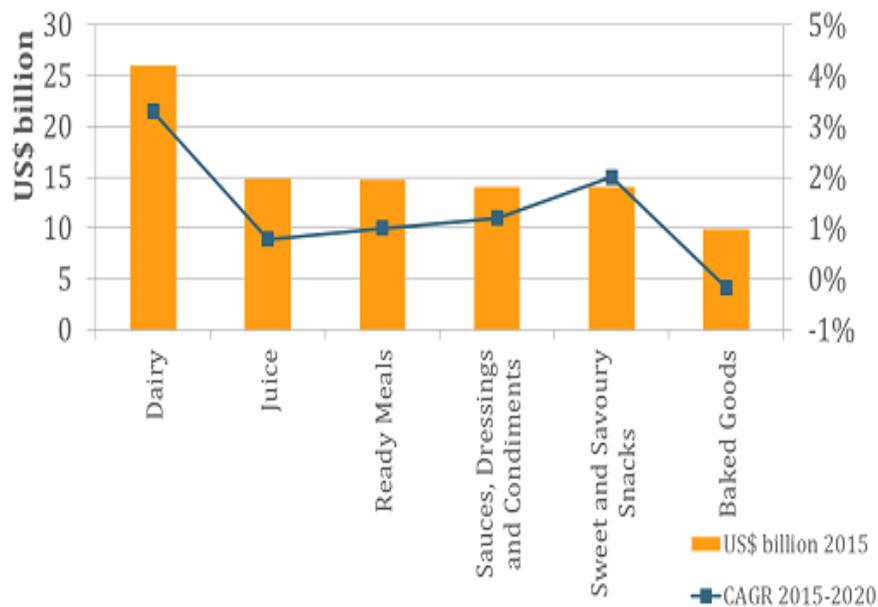


Figure 2. Driving clean label categories in packaged food, soft and hot drinks (Source: [16])

According to Figure 2, in 2015, dairy was the clean label frontrunner with over US\$25 billion in value sales across food and beverages, while a cluster of other categories including juice, ready meals, sauces, dressings and condiments along with sweet and savory snacks bearing clean label claims all accounted for between US\$14-15 billion respectively [16].

According Innova Market Insights, the launches in supplement tracked with clean label claims has grown at an annual combined compound rate of fourteen percent from 2009 to 2013 for three key markets in the Americas: USA, Brazil and Mexico.

More specifically on these markets, the surge in the number of supplements that carry one or more clean label claims is as follows [17]:

- Brazil – Up 125%, from 16% 2010 to 36% in 2014.

- Mexico – Up 110%, from 10% 2010 to 21% in 2014.
- United States – Up 28%, from 46% 2010 to 59% in 2014.

The demand for vegetarian supplements also increased in the last few years to remain a key driver of the clean label movement, this was evident as purchases of 38% of supplement users were reported in 2013, compared to 35% in 2011. As a result, the vegetarian claim appears on 35% of clean label supplements in the United States [17].

Thus, clean label food seems healthier as the majority of consumers think and the reason for is that food and beverages claimed to be natural or contains natural ingredients, as well as being free from additives are better accepted. A more in-depth look at these argumentation shows that Germans are more motivated by no artificial

ingredients and no additives claims than natural and organic. The British, Dutch, Italians and Spanish are more concerned about low fat/sugar/salt than other nationalities, and organic is most appealing to those in Italy, Poland and Turkey. This demonstrates the importance of understanding what is acceptable to local markets in order to influence purchasing decisions [18].

## 2.1. Food Biopreservation

In spite of modern advances in technology, the preservation of foods is still a big issue, not only for developing countries (where the implementation of food preservation technologies are clearly needed) but also for the industrialized world [19]. In the last decades, due to consumer demand for higher quality and naturalness of foods, as well as strict government requirements for guarantees in food safety, the food producers have faced conflicting challenges. Since chemical additives have generally been used to work on specific microorganisms [20].

Thus, over the last few years, the interest in food biopreservation has dramatically increased. Evidences for this movement are shown in the bakery market since the advertisement of the absence of additives/preservatives represented 21% of new bread products launched in Europe in 2013 and 2014 [21].

The enzymes are used in the dairy, bakery, brewing, and protein-modification industries [22]. The reason for the enzyme use in the baking industry has been attributed to the “clean label” appeal. Furthermore, changes in regulations have also promoted the clean label trend, in which the additives and processing aids has been banned or phased out. Where the enzymes are the preferred replacements for these chemical additives [23].

A whole range of enzymes are used in the baking industry where they improve the processing or quality parameters of goods such as bread, buns, rolls, cakes, biscuits, etc. There are many typical effects that can be achieved by using enzymes, such as, extended shelf life, improved dough fermentation, dough machinability and stability, increased loaf volume, finer and whiter crumb structure and intensified crust color [24].

From a regulatory perspective, enzymes are destroyed during baking and, therefore, do not have to be declared on ingredient labels. Those that do remain need only be labeled as “enzymes.” This label terminology is more consumer-friendly because consumers associate enzymes with more natural and nontoxic connotations. These changes in consumer attitudes and regulations will continue to drive innovations in enzyme technologies [23]. Thus, enzymatic processing offers a sustainable, specific bio-processing tool capable to deliver products that are natural, contain a reduced amount of chemicals and present sensorial properties appealing [25].

To ensure the food safety, there is a great interest in biopreservation procedures involving the use of saprophytic microorganisms and/or their metabolites, either to inhibit pathogens or to extend shelf-life. The main bacterial metabolites with potential for use as biopreservatives are the antimicrobial peptides, which are described as bacteriocins and bacteriocin-like inhibitory substances (BLIS), and may be produced by many bacterial species [26].

Biopreservation, is defined as the extension of shelf life and enhanced safety in foods by using natural or controlled microbiota and/or antimicrobial compounds. It is an innocuous and ecological approach to the problem of food preservation and has gained attention recently. Consequently, certain lactic acid bacteria (LAB), with demonstrated antimicrobial properties commonly associated with foods are being studied to increase the safety and/or prolong the shelf life of foods. LAB include the genera *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium*, *Aerococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weisella* [27,28].

Bacteriocins can be introduced into food at least in three different ways: (i) by adding the purified or partially purified bacteriocin directly to the food; (ii) by incorporating an ingredient based on a fermentate of a bacteriocin-producing strain into the food; or (iii) by producing the bacteriocin in site by using the Bacterial strains as starter or protective/adjunct cultures. Alternatively, bacteriocins can be incorporated into packaging materials used to protect foods from external contamination [19,20].

The incorporation of bacteriocins as a biopreservative ingredient into food model systems has been studied extensively and has been shown to be effective in the control of pathogenic and spoilage microorganisms. However, a more practical and economic alternative of incorporating bacteriocins into foods could be the direct addition of the bacteriocin-producing cultures into the food [30].

According to Angiolillo [31] and Bosma, Forster and Nielsen [32] the LAB bacteriocins have many attractive characteristics that make them suitable candidates to use as food preservatives, such as:

- Protein nature, inactivation by proteolytic enzymes of gastrointestinal tract;
- Non-toxic to laboratory animals tested and generally non-immunogenic;
- Inactive against eukaryotic cells
- Generally thermoresistant (can maintain antimicrobial activity after pasteurization and sterilization);
- Broad bactericidal activity affecting most of the Gram-positive bacteria and some, damaged, Gram-negative bacteria including various pathogens such as *L. monocytogenes*, *Bacillus cereus*, *S. aureus*, and *Salmonella*;
- Genetic determinants generally located in plasmid, which facilitates genetic manipulation to increase the variety of natural peptide analogues with desirable characteristics.

## 2.2. ‘Clean Label’ in Bakers Products

The largest bakery product marketed in Europe is bread. It plays a notable role in the diet, with per capita annual consumption of bread in the EU around 62 kg; in Portugal, annual bread consumption stands slightly higher, at 70 kg per capita. Biscuits are the second largest bakery product consumed, followed by industrial pastries/cakes [33,34].

Bakery products are an important part of a balanced diet and, today, a wide variety of those products can be found in the supermarket. However, bakery products, like many processed foods, are exposed to physical, chemical and microbiological spoilage. While physical and chemical

spoilage limits the shelf life of a low and intermediate moisture bakery products, microbiological spoilage by bacteria, yeast and molds is the concern in high moisture products i.e., products with water activity (a.w) >0.85 [35,36,37].

In the baking industry, the most commonly used chemical preservatives to prevent mold spoilage are the following: propionates (calcium or sodium propionate), sorbates (sorbic acid and potassium sorbate), benzoates, parabens (methyl and propyl) and acetic acid. Due to their lack of activity against yeast, propionates are the most widely used antimicrobial in yeast-raised baked foods [38].

The use of chemical preservatives has been rejected, because of the consumer trend towards a preference for natural foods or food products with as few chemical preservatives as possible. This trend is partly driven by the emergence of an increasing number of reports in the public domain which have chemicals in foods associated with the development of diseases [39].

An urge exists in the bakery industry to reduce the quantities of chemical preservatives. The use of natural preservatives would enable bakeries to market the so called 'clean label' or 'label friendly' products [40]. Thus, the "free-from" trend leads the bakery industry to reconsider traditional preservation methods and replace chemical preservatives with natural alternatives to guarantee a clean label [41].

With the popularity of the clean-label products, some bakers are hesitant to add preservatives; the word "preservative" does not stir images or feelings of "homemade." Fortunately, clean-label preservatives for mold inhibition do exist and are being increasingly available [42].

In the bakery product context, bioprotective cultures (i.e. microorganisms exhibiting antifungal activities) represent a growing interest as an alternative to reduce levels or fully eliminate chemical preservatives. The use of lactic acid bacteria (LAB) and propionibacteria as bioprotective cultures is particularly of interest. Indeed, LAB and propionibacteria are naturally present in many fermented foods and have a long history of safe use as starter cultures in the food industry [43].

Thus, the ability of selected lactic acid bacteria to inhibit the growth of rope-forming *Bacillus* strains in laboratory experiments and in wheat bread was investigated. Growth of *Bacillus subtilis* and *Bacillus licheniformis* was inhibited by *Lactobacillus plantarum* VTT E-78076 and *Pediococcus pentosaceus* VTT E-90390 in an automated turbidometry assay and in test bakings. Rope spoilage of wheat bread was inhibited by adding 20–30 g of sourdough/100 g of wheat dough if the sourdough was fermented with *Lactobacillus plantarum* VTT E-78076, *Pediococcus pentosaceus* VTT E-90390 or *Lactobacillus brevis* (commercial starter culture) and the pH values of sourdoughs were adjusted below 4.0 and the amount of total titratable acidity value was >12. Addition of lactic acid alone in concentrations comparable with those formed in sourdoughs did not prevent rope spoilage [44].

The fungicidal activity of Phenyllactic acid (PLA) and growth inhibition by PLA were evaluated by using a microdilution test and 23 fungal strains belonging to 14 species of *Aspergillus*, *Penicillium*, and *Fusarium* that were isolated from bakery products, flours, or cereals. Less than 7.5 mg of PLA ml<sup>-1</sup> was required to obtain 90%

growth inhibition for all strains, while fungicidal activity against 19 strains was shown by PLA at levels of ≤10 mg ml<sup>-1</sup>. Levels of growth inhibition of 50 to 92.4% were observed for all fungal strains after incubation for 3 days in the presence of 7.5 mg of PLA ml<sup>-1</sup> in buffered medium at pH 4, which is a condition more similar to those in real food systems. Under these experimental conditions PLA caused an unpredictable delaying effect that was more than 2 days long for 12 strains, including some mycotoxigenic strains of *Penicillium verrucosum* and *Penicillium citrinum* and a strain of *Penicillium roqueforti* (the most widespread contaminant of bakery products); a growth delay of about 2 days was observed for seven other strains. The effect of pH on the inhibitory activity of PLA and the combined effects of the major organic acids produced by lactic acid bacteria isolated from sourdough bread (PLA, lactic acid, and acetic acid) were also investigated. The ability of PLA to act as a fungicide and delay the growth of a variety of fungal contaminants provides new perspectives for possibly using this natural antimicrobial compound to control fungal contaminants and extend the shelf lives of foods [45].

Rizzello *et al.* [46] investigated the antifungal activity of sourdough fermented (*Lactobacillus plantarum* LB1 and *Lactobacillus rossiae* LB5) wheat germ (SFWG). Preliminarily, methanol and water/salt-soluble extracts from SFWG were assayed by agar diffusion towards *Penicillium roqueforti* DPPMAF1. As shown by hyphal radial growth rate, the water/salt-soluble extract showed the inhibition of various fungi isolated from bakeries. The antifungal activity was attributed to a mixture of organic acids and peptides, which were synthesized during fermentation. Formic acid (24.7 mM) showed the highest antifungal activity. Four peptides, presenting similarities with well-known antifungal sequences, were identified and chemically synthesized. The minimal inhibitory concentration was 2.5–15.2 mg/ml. Slices of bread with the addition of 4% (wt/wt) freeze-dried SFWG were packed in polyethylene bags and stored at room temperature. Slices did not show contamination by fungi until at least 28 days of storage and behaved as the calcium propionate (0.3%, wt/wt).

Ryan *et al.* [47] verify *Lactobacillus amylovorus* DSM19280 has been shown to produce a wide spectrum of antifungal compounds which are active against common bread spoilage fungi. Among the indicator molds, *Aspergillus fumigatus* and *Fusarium culmorum* were the most sensitive organisms. Several antifungal compounds were found to be present in synthetic medium inoculated with *L. amylovorus* DSM19280 strain, some of them being reported for the first time. Wheat doughs fermented with *L. amylovorus* DSM19280 had good rheological properties and the breads thereof were of high quality, as shown by rheofermentometer and texture analyser measurements. The quality of sourdough and bread fermented with *L. amylovorus* DSM 19280 was comparable to that obtained by using *L. amylovorus* DSM20531 T. The biological preservation of bread with *L. amylovorus* DSM 19280 was also compared to the most commonly used antifungal agent Calcium propionate. Breads containing sourdough fermented with *L. amylovorus* DSM 19280 were more effective in extending the shelf life of bread than the calcium propionate.

Table 1. Studies concerning clean label bakery products

Product	Assay	Conclusion	References
Bread	Investigation of the ability of selected lactic acid bacteria to inhibit the growth of rope-forming <i>Bacillus strains</i> in laboratory experiments and in wheat bread.	Rope spoilage of wheat bread was inhibited by adding 20–30 g of sourdough per 100 g of wheat dough when the sourdough was fermented with <i>Lactobacillus plantarum</i> VTT E-78076, <i>Pediococcus pentosaceus</i> VTT E-90390 or <i>Lactobacillus brevis</i> (commercial starter culture). Addition of lactic acid alone in concentrations comparable with those formed in sourdoughs did not prevent rope spoilage.	[44]
Bakery products, flours or cereals.	The fungicidal activity of Phenyllactic acid (PLA) and growth inhibition by PLA were evaluated by using a microdilution test and 23 fungal strains belonging to 14 species of <i>Aspergillus</i> , <i>Penicillium</i> , and <i>Fusarium</i> that were isolated from bakery products, flours or cereals.	The ability of PLA to act as a fungicide and delay the growth of a variety of fungal contaminants provides new perspectives for possibly using this natural antimicrobial compound to control fungal contaminants and extend the shelf lives of foods.	[45]
Slices of bread	Analysis of the antifungal activity of sourdough fermented ( <i>Lactobacillus plantarum</i> LB1 and <i>Lactobacillus rossiae</i> LB5) wheat germ (SFWG).	Formic acid (24.7 mM) showed the highest antifungal activity. The minimal inhibitory concentration was 2.5–15.2 mg/ml. Slices did not show contamination by fungi until at least 28 days of storage and behaved as the calcium propionate (0.3%, wt/wt).	[46]
Bread	Verification of the ability of the <i>Lactobacillus amylovorus</i> DSM19280 to produce a wide spectrum of antifungal compounds which are active against common bread spoilage fungi.	The biological preservation of bread with <i>L. amylovorus</i> DSM 19280 was also compared to the most commonly used antifungal agent Calcium propionate. Breads containing sourdough fermented with <i>L. amylovorus</i> DSM 19280 were more effective in extending the shelf life of bread than the calcium propionate.	[47]
Bread	Analysis of the conservation of a semi-liquid bio-preserver (SL778) developed with <i>Lactobacillus plantarum</i> CRL 778, a lactic acid bacterium (LAB) having antifungal activity.	No undesirable difference was detected between bread control without SL778 and bread manufactured with SL778 (stored at 4 or –20 °C). The SL778 semi-liquid bio-preserver can be stored at 4 or –20 °C without modifying its antifungal activity during 14 days.	[48]
Bread	Investigation of the antifungal activity of <i>Lactobacillus amylovorus</i> DSM19280 as a starter culture for gluten-free quinoa sourdough bread under pilot-plant conditions to extend the microbial shelf life.	The combination of quinoa flour fermented with the antifungal <i>L. amylovorus</i> DSM19280 serves as a great potential biopreservative ingredient to produce gluten-free breads with an improved nutritional value, better bread quality and higher safety due to an extended shelf life therefore meeting consumer needs for good quality and preservative-free food products.	[21]
Bread and panettone	Two hundred and sixteen LAB cultures from sourdoughs and dough for bread and panettone production were screened for in vitro antifungal properties against three indicator cultures ascribed to <i>Aspergillus japonicus</i> , <i>Eurotium repens</i> , and <i>Penicillium roseopurpureum</i> , isolated from bakery environment and moldy panettone.	The use of sourdough as a baking improver combined with baker's yeast also allows replacing additives by “clean label” ingredients in industrial baking. Moreover, the fermentation of sourdough in the bakery industry is an alternative to the use of additives.	[49]
Bread	Assessment of the potential of commercially available ‘clean label’ fermentates to replace the preservative function of propionate in bread.	The antifungal activity of the fermentates was greater the lower the pH of the growth medium. Additionally, <i>P. paneum</i> was in general more tolerant to the fermentates than <i>P. chrysogenum</i> . The challenge and sensorial tests showed that bread prepared with FA (1.3%), FB (2%) and FC (1.3 and 2%) were as microbiologically stable and sensorially acceptable as the reference. Therefore, these fermentates can be used as ‘label friendly’ preservatives in bread products.	[39]
Bread	Evaluation of the antifungal performance of three different <i>Lactobacillus</i> species.	The chemical application of the antifungal carboxylic acids succeeded in a longer shelf life than achieved only by acidifying the dough (+25 %), which is an evidence of their contribution to the antifungal activity and their synergy.	[50]
Cake	The fat in a sponge cake formulation was partially replaced (0%, 30%, 50% and 70%) with OptiSol™5300. This natural functional ingredient derived from flax seeds, rich in fiber and alpha-linoleic acid, provides a natural substitute for guar and xanthan gums, avoiding E-numbers on labels.	There were no significant differences ( $P > 0.05$ ) in texture, flavor and overall acceptance between the control and the 30% substitution cake, nor in the rapidly digestible starch values. Consequently, replacing up to 30% of the fat with OptiSol™5300 gives a new product with health benefits and a clean label that resembles the full-fat sponge cake.	[51]
Sourdough breads	Sourdough breads were prepared with different sourdoughs from <i>G. albidus</i> and <i>K. baliensis</i> strains (24, 30 and 48 hours of fermentation, respectively) and analyzed regarding bread volume, crumb hardness and sensory characteristics.	The positive effects of levan were masked to a certain extent by the impact of the natural acidification during fermentations. While levan-producing acetic acid bacteria are a promising alternative for the development of clean-label gluten-free breads without the need of additives, an appropriate balance between acidification and levan production (amount and structure) must be reached.	[52]
Biscuits	The study focused on the use of fennel and chamomile extracts, rich in phenolic compounds, as natural antioxidants in biscuits. It compared their performance with a synthetic antioxidant widely used, the butylated hydroxyl anisole (BHA).	Both natural and synthetic additives conferred similar antioxidant activity to the biscuits. Therefore, natural additives are a more convenient solution for consumers who prefer foods that are “free” from synthetic additives. Additionally, natural additives were obtained by aqueous extraction, an environmentally friendly and safe process.	[53]

This study had the purpose of analyzing the conservation of a semi-liquid bio-preserver (SL778) developed with *Lactobacillus plantarum* CRL 778, a lactic acid bacterium (LAB) having antifungal activity. In addition, a synergistic effect was observed when SL778 and CPa were added to the bread formulation. SL778 maintained its efficacy on bread preservation up to 14-day storage at both temperatures. However, the decrease in cell viability and lactic acid concentration of the starter at  $-20^{\circ}\text{C}$  might have affected the effectiveness of SL778. Sensory attributes (acidic and spicy tastes and acidic smell) of breads manufactured with starter SL778 (stored at 4 or  $-20^{\circ}\text{C}$ ) were evaluated. No undesirable difference was detected regarding bread control without SL778 and bread manufactured with SL778 (stored at 4 or  $-20^{\circ}\text{C}$ ). Moreover, LAB acidification in wheat fermentation presents other beneficial properties, for example: (i) it creates an optimum pH for the activity of endogenous proteinases, which improves texture changes and flavor and (ii) it delays starch retrogradation and bread firming. In conclusion, the SL778 semi-liquid bio-preserver can be stored at 4 or  $-20^{\circ}\text{C}$  without modifying its antifungal activity during 14 days [48].

Axel *et al.* [21] investigated the antifungal activity of *Lactobacillus amylovorus* DSM19280 as a starter culture for gluten-free quinoa sourdough bread under pilot-plant conditions to extend the microbial shelf life. Challenge tests against environmental molds were conducted and a negative control with non-antifungal strain, *L. amylovorus* DSM20531T, as well as a chemically acidified and a non-acidified control, were included. The application of quinoa sourdough fermented with the antifungal *L. amylovorus* DSM19280 extended the mold-free shelf life by 4 days compared to the non-acidified control. No significant difference in lactic acid production was found between the *Lactobacilli* strains. The concentration of 4-hydroxyphenyllactic acid, phloretic acid, 3-phenyllactic acid and hydroferulic acid were significantly higher ( $P < 0.01$ ) in the quinoa sourdough fermented with the antifungal *L. amylovorus* DSM19280 when compared to the non-antifungal strain, thus indicating their contribution to the antifungal activity. Evaluation of bread characteristics such as specific volume or crumb hardness, revealed that the addition of *L. amylovorus* fermented sourdough also improved bread quality. In conclusion, the combination of quinoa flour fermented with the antifungal *L. amylovorus* DSM19280 serves as a great potential biopreservative ingredient to produce gluten-free breads with an improved nutritional value, better quality and higher safety due to an extended shelf life, therefore meeting consumer needs for good quality and preservative-free food products.

Two hundred and sixteen LAB cultures from sourdoughs and dough for bread and panettone production were screened for in vitro antifungal properties against three indicator cultures ascribed to *Aspergillus japonicus*, *Eurotium repens*, and *Penicillium roseopurpureum*, isolated from bakery environment and moldy panettone. Nineteen preselected isolates were subjected to determination of the minimum inhibitory concentration against the indicator cultures. Sourdoughs prepared with the two most promising strains, identified as *Lactobacillus rossiae* LD108 and *Lactobacillus paralimentarius* PB127, were characterized. The sourdough extracts were subjected to

HPLC analysis coupled with a microtiter plate bioassay against *A. japonicus* to identify the active fractions. MALDI-TOF MS analysis revealed the occurrence of a series of peptides corresponding to wheat  $\alpha$ -gliadin proteolysis fragments in the active fraction from the *L. rossiae* LD108 sourdough. The ability to prevent mold growth on bread was demonstrated for both strains, whereas *L. rossiae* LD108 also inhibited mold growth on panettone [49]. Thus, the use of sourdough in baked and steamed bread production worldwide is increasing due to the improved sensory and nutritional quality of sourdough bread when compared to bread produced by straight dough processes. Traditionally, sourdough has been used as a leavening agent. Sourdough microbiota comprises yeasts and lactic acid bacteria. The use of sourdough as a baking improver combined with baker's yeast also allows replacing additives by "clean label" ingredients in industrial baking. Moreover, the fermentation of sourdough in the bakery industry is an alternative to the use of additives [50].

Samapundo *et al.* [40] assessed the potential of commercially available 'clean label' fermentates to replace the preservative function of propionate in bread. This study was performed in two parts. In the first part, the inhibitory activities of selected fermentates, FA (cultured corn syrup and citric acid), FB (cultured wheat solids), FC (cultured dextrose) and FD (cultured dextrose), towards *Penicillium chrysogenum* and *Penicillium paneum* were assessed as a function of pH (4.5–6.5) on malt extract agar (MEA). In the second part, challenge, shelf life and sensorial tests were used to determine the suitability of these fermentates to replace calcium propionate in bread. All the fermentates evaluated had appreciable inhibitory activities towards *P. chrysogenum* and *P. paneum*. The antifungal activity of the fermentates was greater the lower the pH of the growth medium. Additionally, *P. paneum* was in general more tolerant to the fermentates than *P. chrysogenum*. The challenge and sensorial tests showed that bread prepared with FA (1.3%), FB (2%) and FC (1.3% and 2%) were as microbiologically stable and sensorially acceptable as the reference. Therefore, these fermentates can be used as 'label friendly' preservatives in bread products.

This study was undertaken to assess the antifungal performance of three different *Lactobacillus* species. Experiments were conducted in vitro and in situ to extend the shelf life of wheat bread. Overall, the strains showed good inhibition in vitro against the indicator mold *Fusarium culmorum* TMW4.2043. Sourdough bread fermented with *Lactobacillus amylovorus* DSM19280 performed best in the in situ shelf life experiment. An average shelf life extension of six more mold-free days was reached when compared to the non-acidified control bread. A range of antifungal-active acids like 3-phenyllactic acid, 4-hydroxyphenyllactic acid and 2-hydroxyisocaproic acid in quantities between 0.1 and 360 mg/kg were present in the freeze-dried sourdoughs. Their concentration differed greatly amongst the species. In particular, although *Lb. reuteri* R29 produced the highest total concentration of these active compounds in the sourdough, its addition to bread did not result in a longest shelf life. However, the chemical application of the antifungal carboxylic acids succeeded in a longer shelf

life than achieved only by acidifying the dough (+25 %), which is an evidence of their contribution to the antifungal activity and their synergy [51].

The fat in a sponge cake formulation was partially replaced (0%, 30%, 50% and 70%) with OptiSol™5300. This natural functional ingredient derived from flax seeds, rich in fiber and alpha-linoleic acid, provides a natural substitute for guar and xanthan gums, avoiding E-numbers on labels. The structure and some physicochemical properties of the formulations were examined, sensory analysis was conducted and changes in starch digestibility due to adding this ingredient were determined. The increase in quantities of OptiSol™5300 led to harder cakes, with less weight loss during baking, without affecting the final cake height. There were no significant differences ( $P > 0.05$ ) in texture, flavor and overall acceptance between the control and the 30% substitution cake, nor in the rapidly digestible starch values. Consequently, replacing up to 30% of the fat with OptiSol™5300 gives a new product with health benefits and a clean label that resembles the full-fat sponge cake [52].

Buckwheat sourdoughs supplemented with molasses as natural sucrose source were fermented with levan-producing *Gluconobacter* (G.) *albidus* TMW 2.1191 and *Kozakia* (K.) *baliensis* NBRC 16680. Sourdough breads were prepared with different sourdoughs from both strains (24, 30 and 48 hours of fermentation, respectively) and analyzed with respect to bread volume, crumb hardness and sensory characteristics. During fermentation, levan, acetic and gluconic acids were increasingly produced, while spontaneously co-growing lactic acid bacteria additionally formed acetic and lactic acids. Sourdoughs from both strains obtained upon 24 hours of fermentation significantly improved the bread sensory properties and quality, including higher specific volume as well as lower crumb hardness. Buckwheat doughs containing isolated levan, with similar molecular size and mass compared to in situ produced levan, in the sourdough for 48 hours, made it possible to verify the positive effect of levan on bread quality. While levan-producing acetic acid bacteria are a promising alternative for the development of clean-label gluten-free breads without the need of additives, an appropriate balance between acidification and levan production (amount and structure) must be reached [53].

The study realized by Caleja *et al.* [54] focused on the use of fennel and chamomile extracts, rich in phenolic compounds, as natural antioxidants in biscuits. It compared their performance with a synthetic antioxidant widely used, the butylated hydroxyl anisole (BHA). The complete nutritional profile, free sugars, fatty acids and antioxidant activity were determined immediately after baking and also after 15, 30, 45 and 60 days of storage. The results showed that the incorporation of natural and synthetic additives did not cause significant changes in color or in nutritional value of biscuits when compared with control samples. Both natural and synthetic additives conferred similar antioxidant activity to the biscuits. Therefore, natural additives are a more convenient solution for consumers who prefer foods that are “free” from synthetic additives. Additionally, natural additives were obtained by aqueous extraction, an environmentally friendly and safe.

Considering all that was presented, bread manufacturers have been increasingly producing the so-called “clean label products” which fit this healthier lifestyle of customers. Food labels of such products have claims such as “no preservatives” or “natural” [41]. Thus the inclusion of 'clean label' continues to drive health-focused new product developments. One can imagine that in the not too distant future, Clean Label will be not the target but the minimally acceptable base for an ingredient declaration.

### 3. Conclusion

Clean label has moved past being a trend. Clean label refers to industry responses to consumer demand for more natural foods and drinks, being that the term “clean label” it incorporates the concept of transparency. The demand for clean labeling has brought the need for clear labeling, resulting in a move to clearer and simpler claims and packaging for maximum transparency.

However, such minimally processed food without chemical preservatives or other artificial additives should still be of high-quality and have an extended shelf life. Thus, this trend leads the food industry to reconsider traditional preservation methods and replace chemical preservatives with natural alternatives to guarantee a clean label.

Consumer awareness of food ingredients and the desire for simple, natural foods have pushed food manufacturers to develop products with clean labels. It is therefore critical to consider the implications of developing a clean-label product, considering the effects of those changes may have on sensory quality and microbiological control, while also maintaining regulatory compliance.

One can imagine that soon, Clean Label will be not the target but the minimally acceptable base for an ingredient declaration.

Another important question is commercializing food for customers with specific dietary needs that has additional challenges for developing products that are standardized. One example would be identifying “clean label” ingredients which can deliver against the taste, sensorial, and shelf life challenges associated with low fat, low carbohydrate, and low sodium food.

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